# An Asymptotic Model of Electroporation-Mediated Molecular Delivery in Skeletal Muscle Tissue

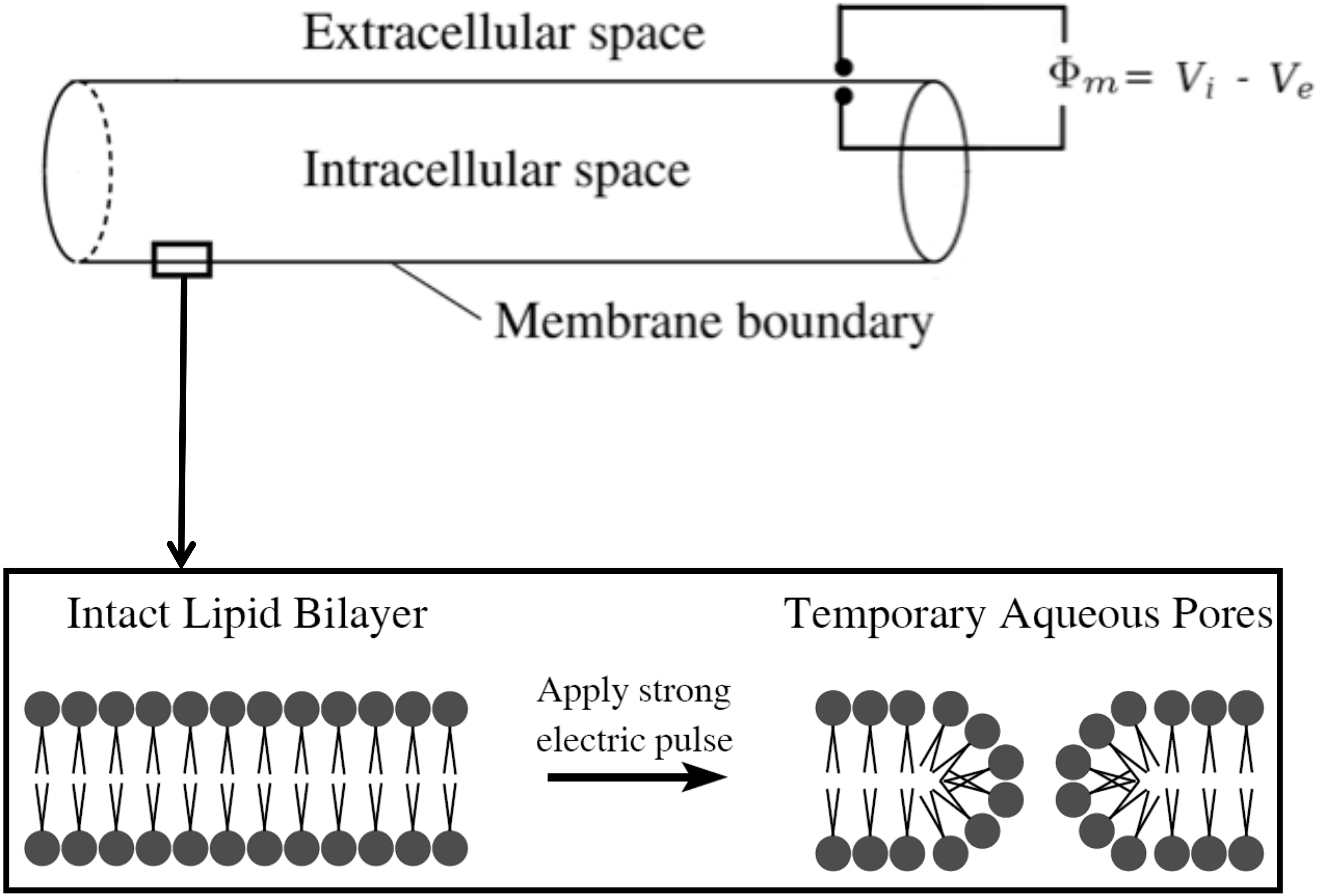
Jonathan Preston Cranford

CASIS Workshop

Livermore, CA

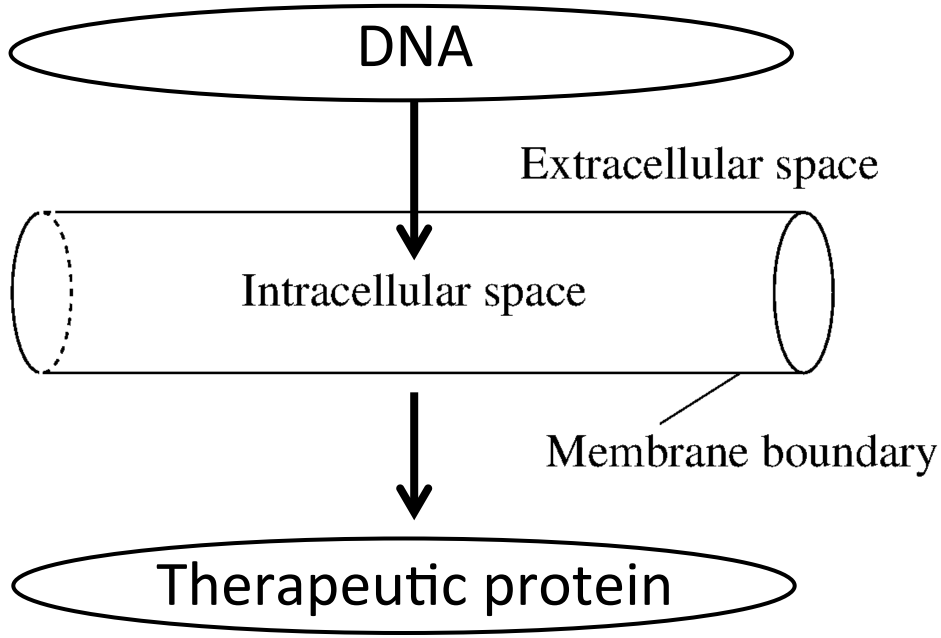
Wednesday May 18, 2016

# Concept of Electroporation (EP)



# Clinical Significance and Experiments

## Gene therapy




EP enhances uptake and expression several orders of mag.!

Challenge: mechanism not well understood

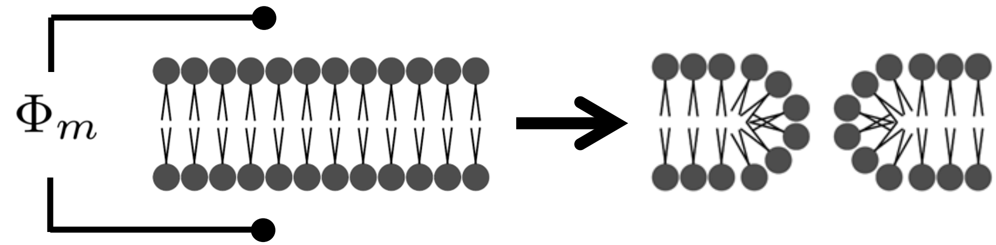
- EP depends on type of tissue and molecule
- Sensitive to pulsing protocol

# Theoretical Studies

## Four components

1. Membrane level

   Creation of pores in response to $\Phi_m$



# Theoretical Studies

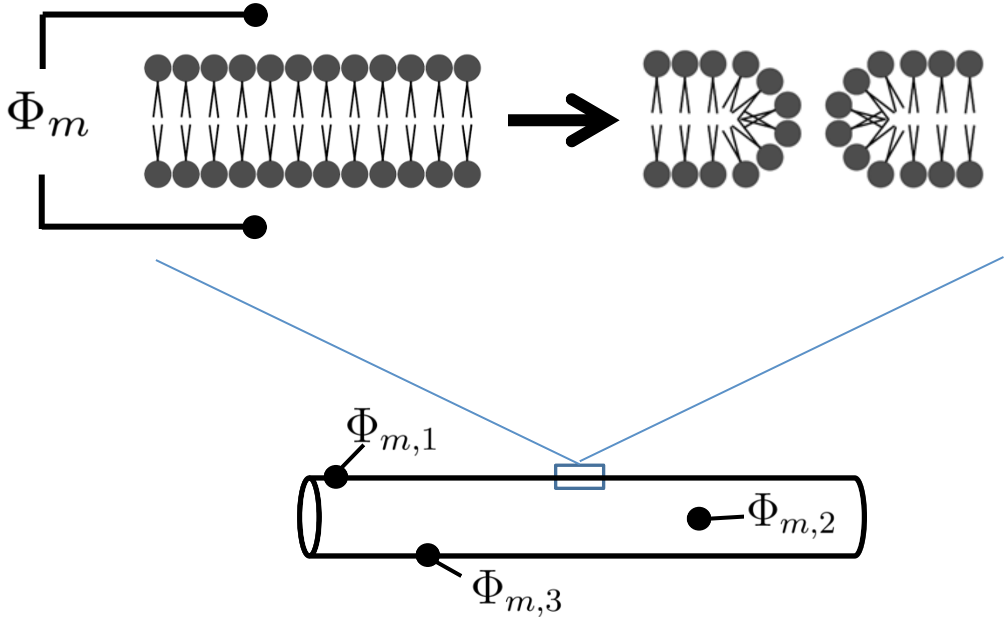
## Four components

1. Membrane level

   Creation of pores in response to $\Phi_m$

2. Cellular level

   Distribution of $\Phi_m$ due to microscale geometry of individual cells



# Theoretical Studies

## Four components

1. Membrane level

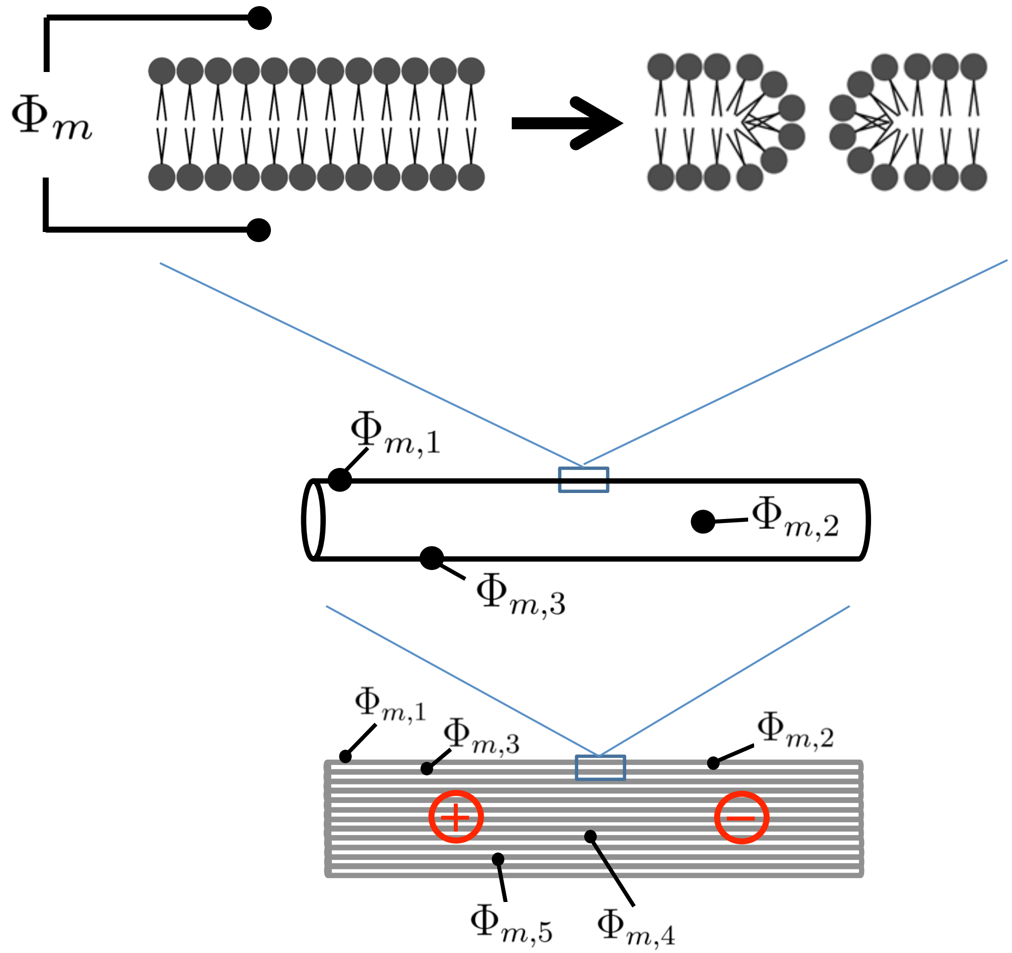
   Creation of pores in response to $\Phi_m$

2. Cellular level

   Distribution of $\Phi_m$ due to microscale geometry of individual cells

3. Tissue level

   Connects macroscale tissue distribution of potential from electric stimulus to $\Phi_m$ at cellular level



# Theoretical Studies

## Four components

1. Membrane level

   Creation of pores in response to $\Phi_m$

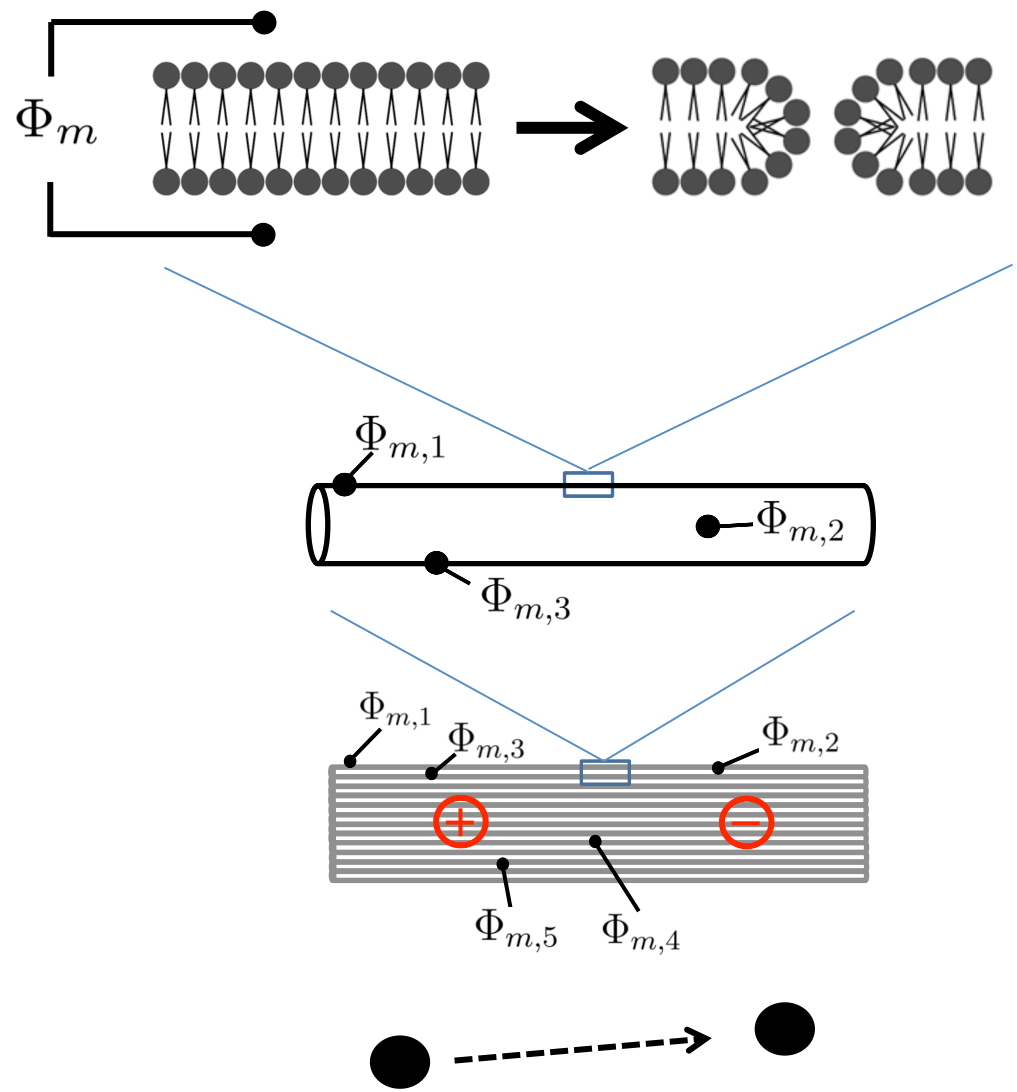
2. Cellular level

   Distribution of $\Phi_m$ due to microscale geometry of individual cells

3. Tissue level

   Connects macroscale tissue distribution of potential from electric stimulus to $\Phi_m$ at cellular level

4. Mass transport

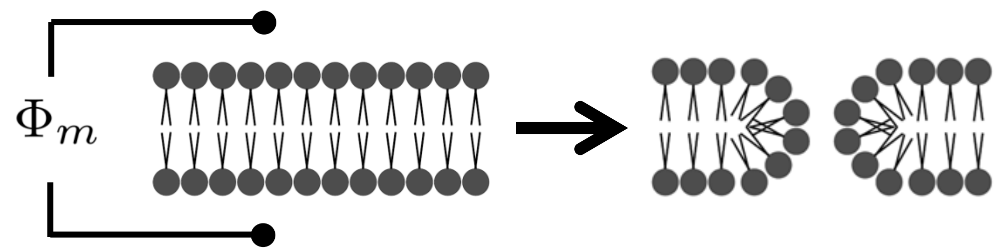


# Theoretical Studies

## Four components

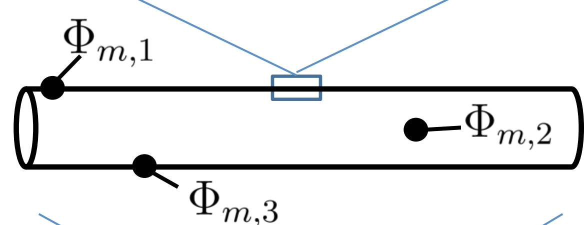
1. Membrane level

   Creation of pores in response to $\Phi_m$



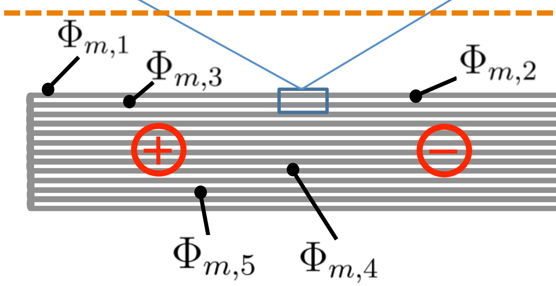

2. Cellular level

   Distribution of $\Phi_m$ due to microscale geometry of individual cells

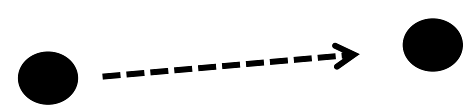



3. Tissue level

   Connects macroscale tissue distribution of potential from electric stimulus to $\Phi_m$ at cellular level



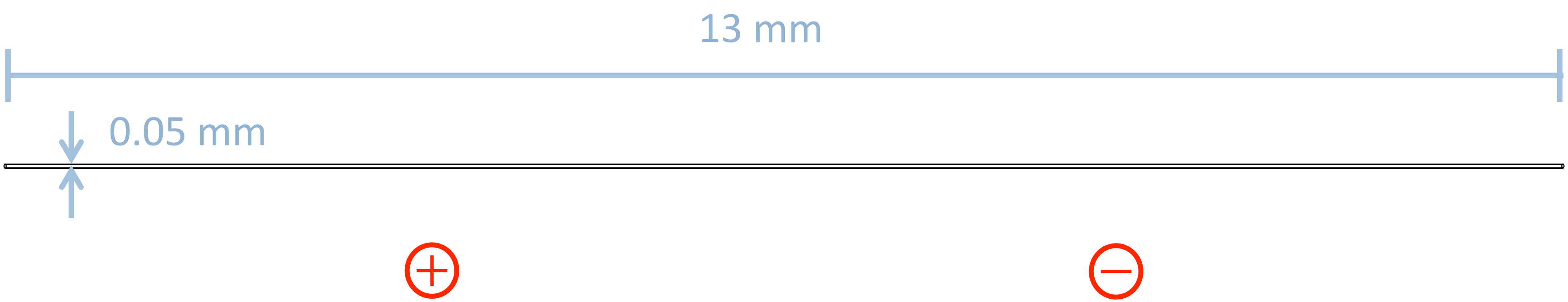

4. Mass transport

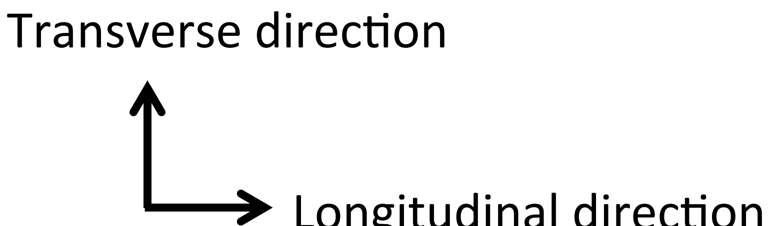


# Theoretical Studies

## Cellular level

Distribution of $\Phi_m$ due to microscale geometry of individual fibers




**Neuron modeling community:**
**Longitudinal component is indispensable!**

**Electroporation modeling community:**
**Longitudinal component is neglected**

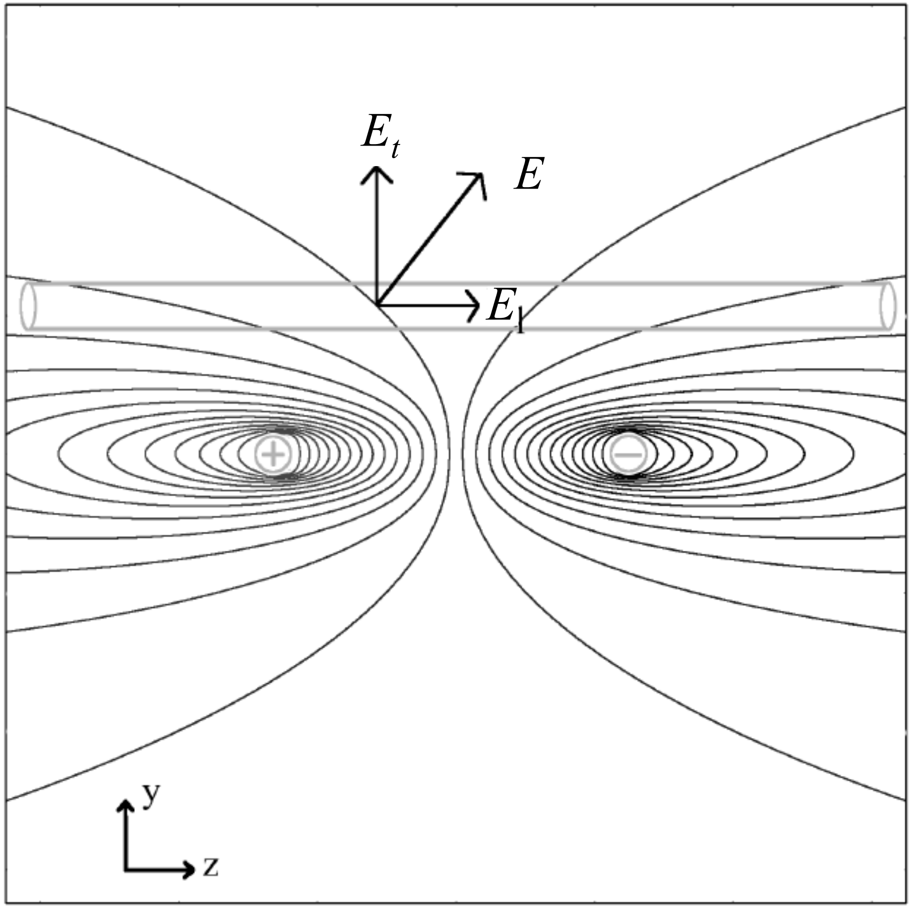
# Theoretical Studies

Cellular level

Distribution of $\Phi_m$ due to microscale geometry of individual cells

EP community models

- $|\Phi_m| \propto |E|$



# Theoretical Studies

Cellular level

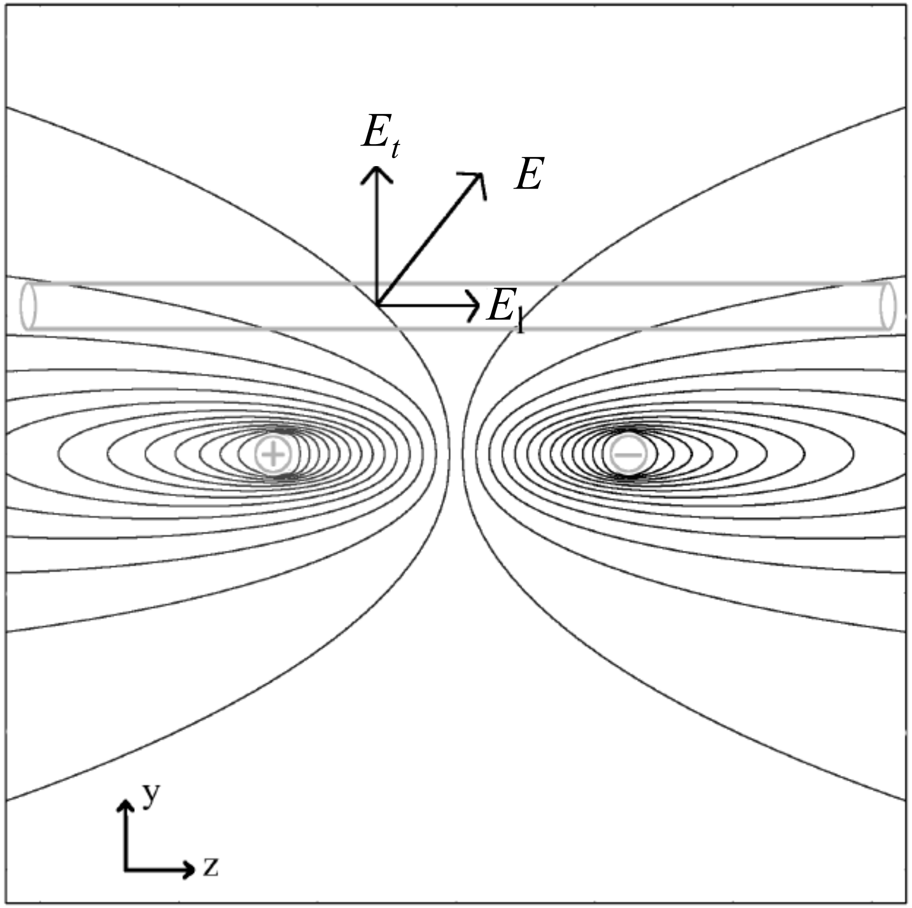
Distribution of $\Phi_m$ due to microscale geometry of individual cells

EP community models

- $|\Phi_m| \propto |E|$

Neuron community models

- "transverse charging"
  $|\Phi_m| \propto |E_t|$
- "longitudinal charging"
  $|\Phi_m| \propto \left|\frac{\partial E_l}{\partial z}\right|$
- $\left|\frac{\partial E_l}{\partial z}\right|$ varies by 7 orders of mag.




**Effect of longitudinal charging on EP not characterized**

# Derivation of Asymptotic Fiber Model

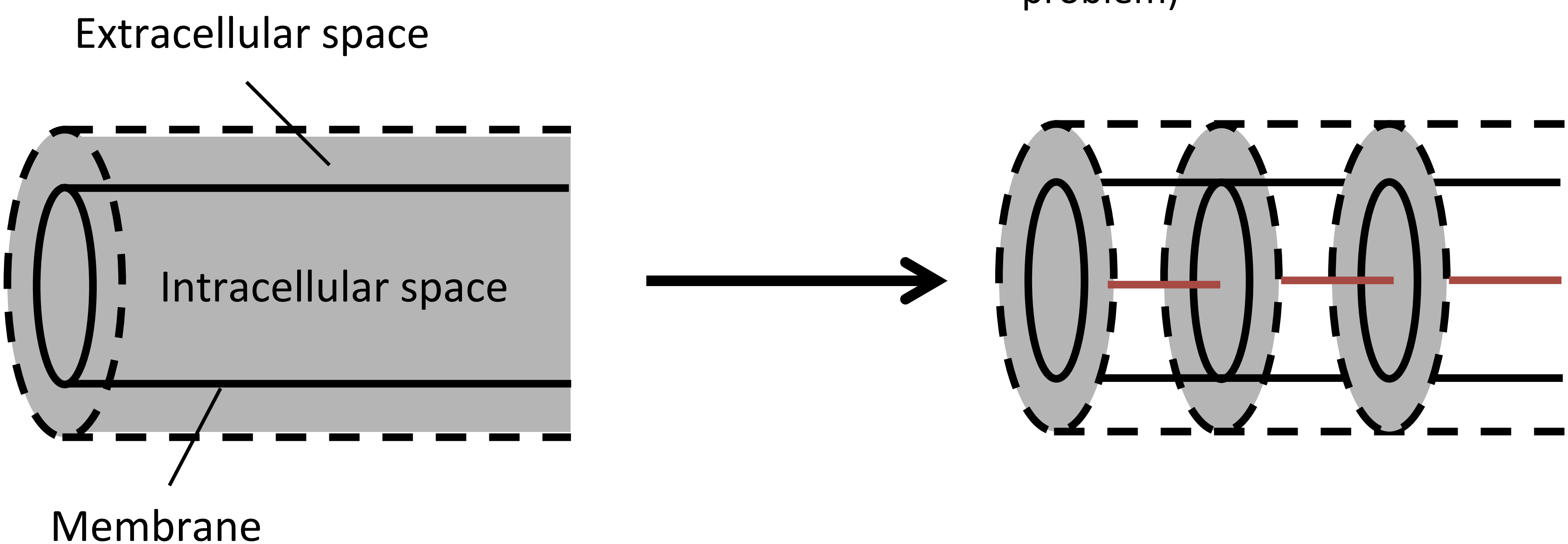
**Purpose: Compute distribution of electric potential in fiber**

Full Fiber Model

(3D BVP)

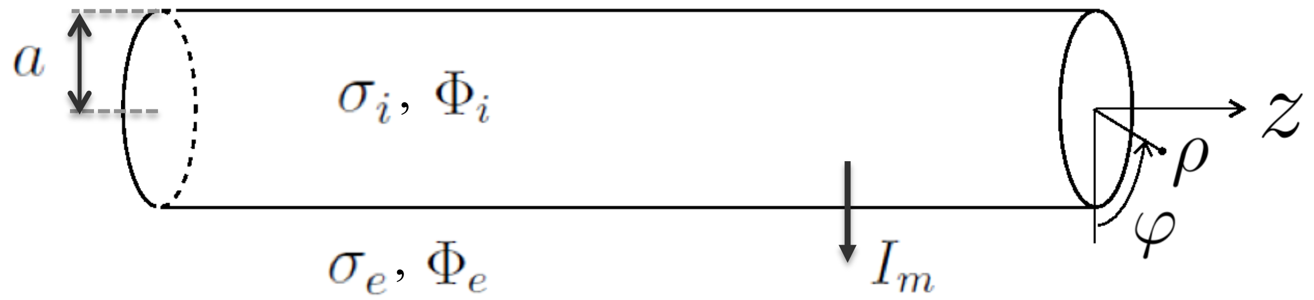
Asymptotic Fiber Model[1]

(series of 2D transverse BVPs connected by 1D longitudinal problem)

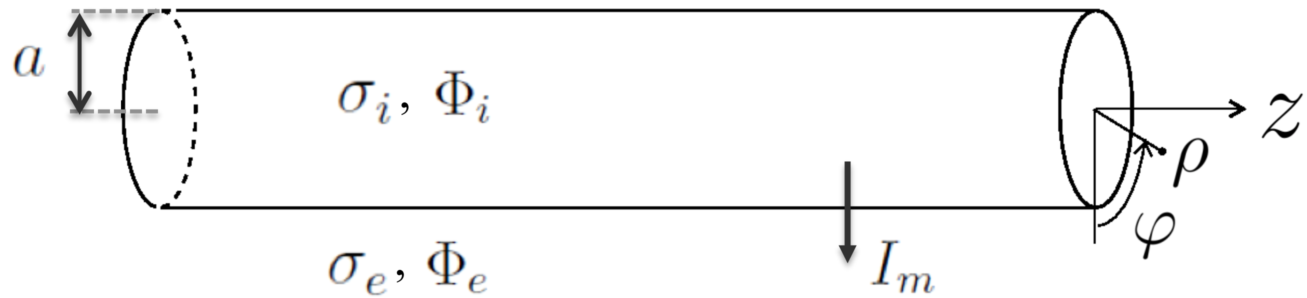



[1] *Cranford, J.P., Kim, B.J., Neu, W.K. (2012) MBEC, 50, 243-251*

# Derivation of Asymptotic Fiber Model



# Derivation of Asymptotic Fiber Model




Scaled governing equations in dimensionless form

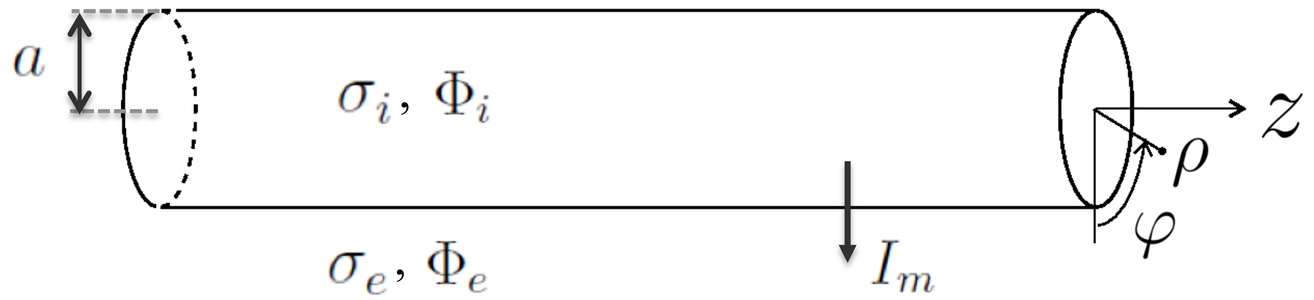
$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\Phi_i}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\Phi_i}{\partial\varphi^2}+\epsilon\frac{\partial^2\Phi_i}{\partial z^2}=0 \qquad \text{in } \rho<a,$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\Phi_e}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\Phi_e}{\partial\varphi^2}+\epsilon\frac{\partial^2\Phi_e}{\partial z^2}=0 \qquad \text{in } \rho>a,$$

$$\frac{\partial\Phi_i}{\partial\rho}=-\frac{\partial\Phi_m}{\partial\tau}-\epsilon\frac{\partial\Phi_m}{\partial T_s}-I_m-\frac{\partial\Psi}{\partial\rho} \qquad \text{on } \rho=a$$

$$\frac{\partial\Phi_e}{\partial\rho}=-\frac{\sigma_i}{\sigma_e}\left\{\frac{\partial\Phi_m}{\partial\tau}+\epsilon\frac{\partial\Phi_m}{\partial T_s}+I_m\right\}-\frac{\partial\Psi}{\partial\rho} \qquad \text{on } \rho=a$$

# Derivation of Asymptotic Fiber Model




Scaled governing equations in dimensionless form

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho \frac{\partial \Phi_i}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \Phi_i}{\partial \varphi^2} + \boxed{\epsilon \frac{\partial^2 \Phi_i}{\partial z^2}} = 0 \qquad \text{in } \rho < a,$$

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho \frac{\partial \Phi_e}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \Phi_e}{\partial \varphi^2} + \boxed{\epsilon \frac{\partial^2 \Phi_e}{\partial z^2}} = 0 \qquad \text{in } \rho > a,$$

$$\frac{\partial \Phi_i}{\partial \rho} = -\frac{\partial \Phi_m}{\partial \tau} - \boxed{\epsilon \frac{\partial \Phi_m}{\partial T_s}} - I_m - \frac{\partial \Psi}{\partial \rho} \qquad \text{on } \rho = a$$

$$\frac{\partial \Phi_e}{\partial \rho} = -\frac{\sigma_i}{\sigma_e}\left\{\frac{\partial \Phi_m}{\partial \tau} + \boxed{\epsilon \frac{\partial \Phi_m}{\partial T_s}} + I_m\right\} - \frac{\partial \Psi}{\partial \rho} \qquad \text{on } \rho = a$$

# Derivation of Asymptotic Fiber Model

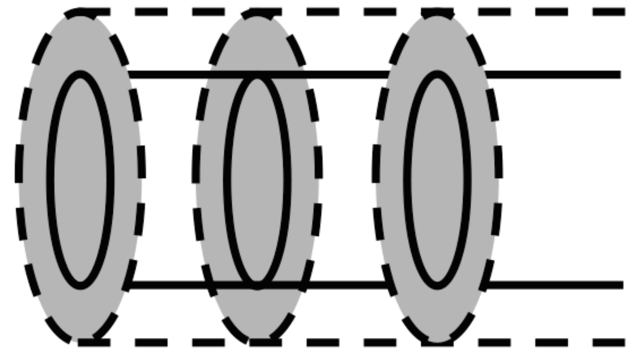
Mean-free equations of the transverse problem

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_i^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_i^0}{\partial\varphi^2}=0 \qquad \text{in } \rho<a$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_e^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_e^0}{\partial\varphi^2}=0 \qquad \text{in } \rho>a$$

$$\sigma_i\frac{\partial\phi_i^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_i\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$

$$\sigma_e\frac{\partial\phi_e^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_e\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$



# Derivation of Asymptotic Fiber Model

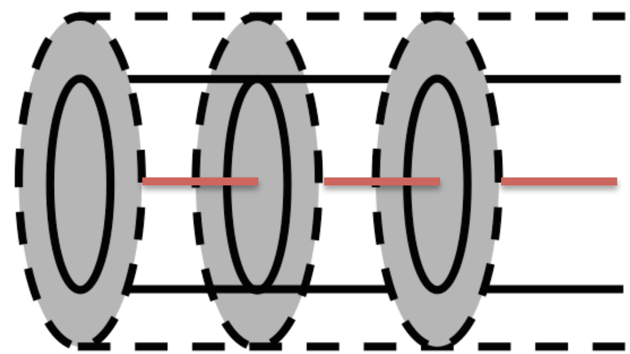
Mean-free equations of the transverse problem

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_i^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_i^0}{\partial\varphi^2}=0 \qquad \text{in } \rho<a$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_e^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_e^0}{\partial\varphi^2}=0 \qquad \text{in } \rho>a$$

$$\sigma_i\frac{\partial\phi_i^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_i\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$

$$\sigma_e\frac{\partial\phi_e^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_e\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$

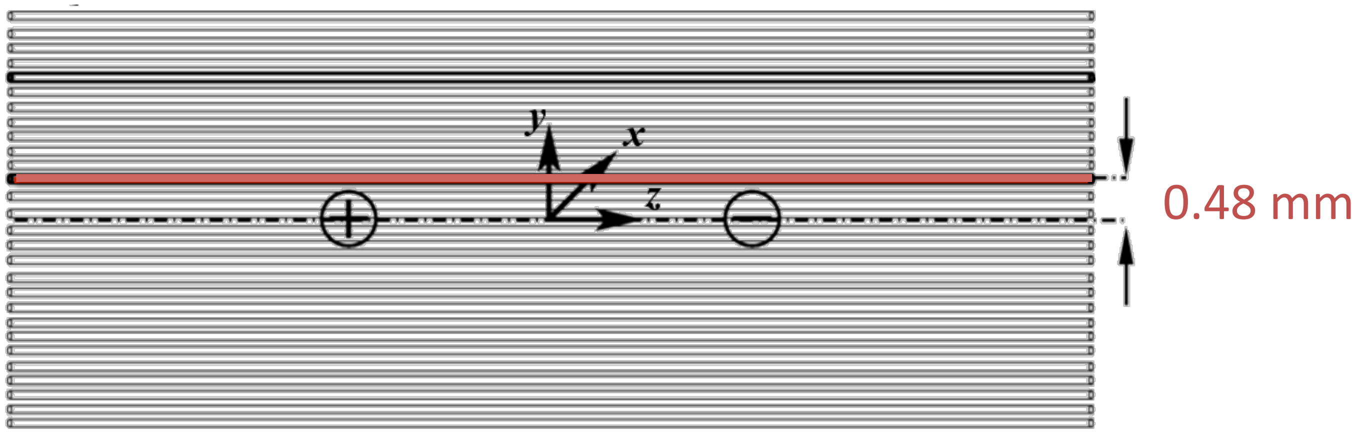


Mean equation of the longitudinal problem

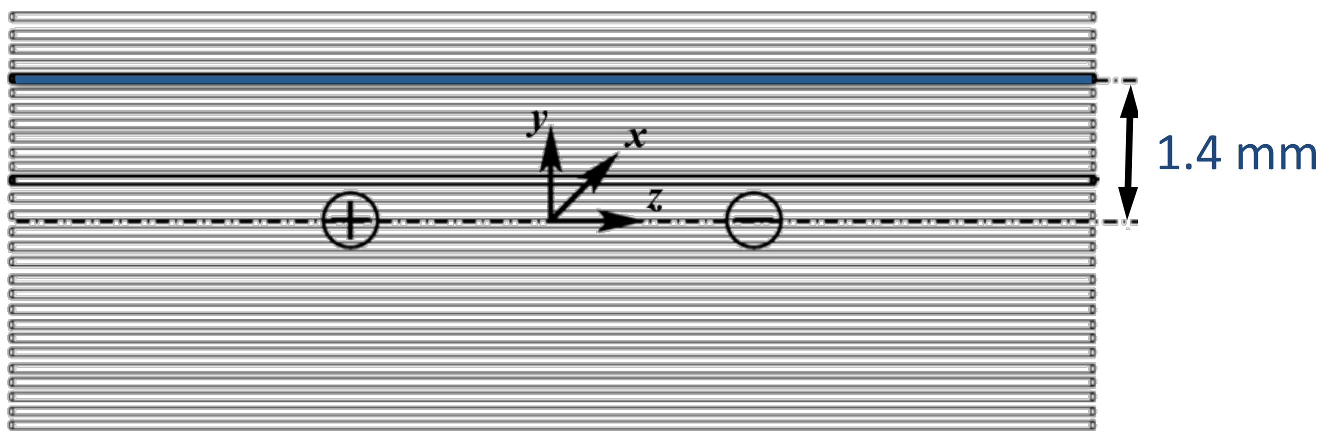
$$\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}=C_m\frac{\partial f_m^0}{\partial t}+\overline{I_m}-\frac{\sigma_i a}{2}\frac{\partial^2\left\langle\Psi\right\rangle}{\partial z^2}$$

# Uptake Results Graphical Aid

Close Fiber

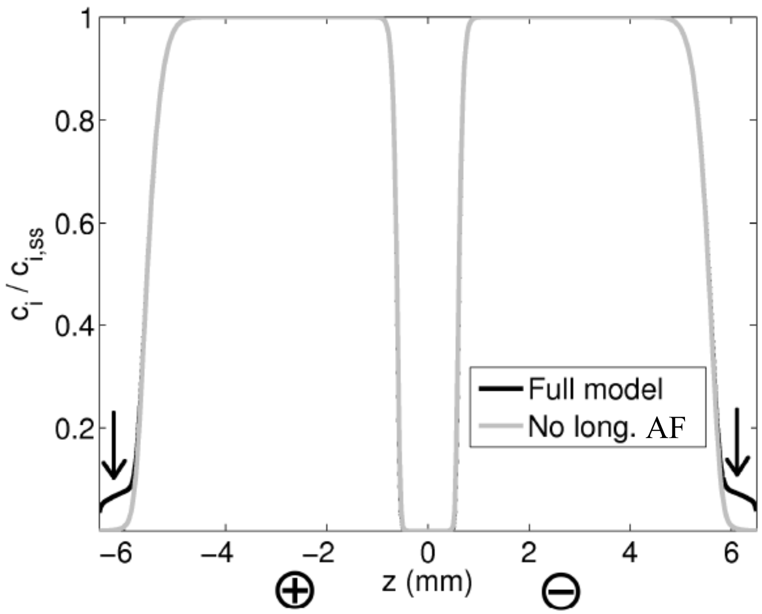



Far Fiber



# Effect of Longitudinal Charging

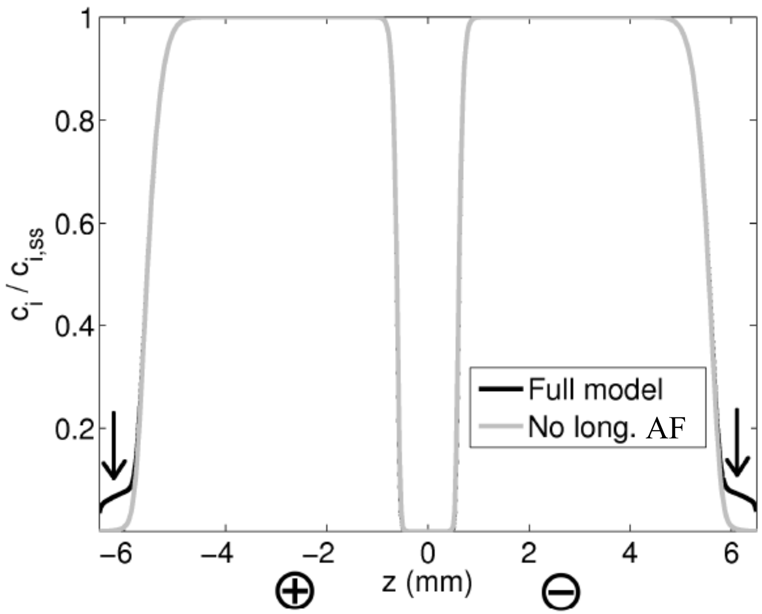
Close Fiber




Longitudinal charging: 0.91 %

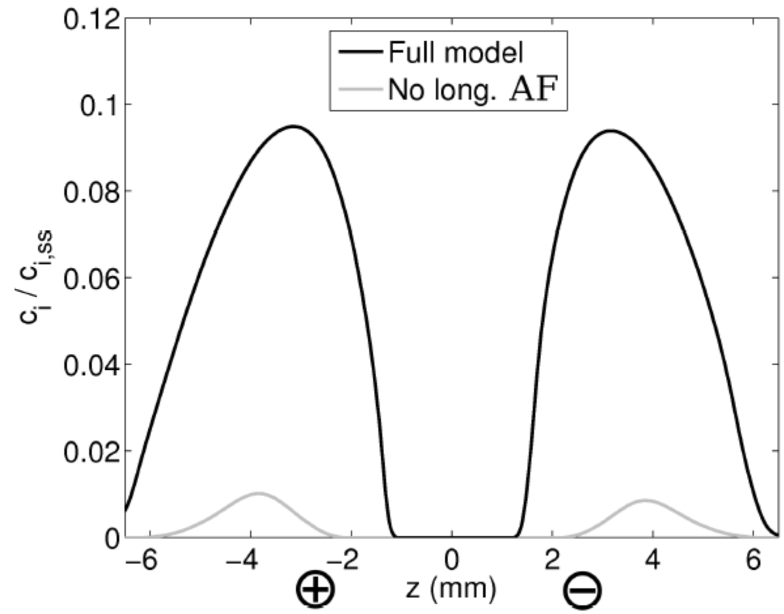
# Effect of Longitudinal Charging

## Close Fiber




Longitudinal charging: 0.91 %

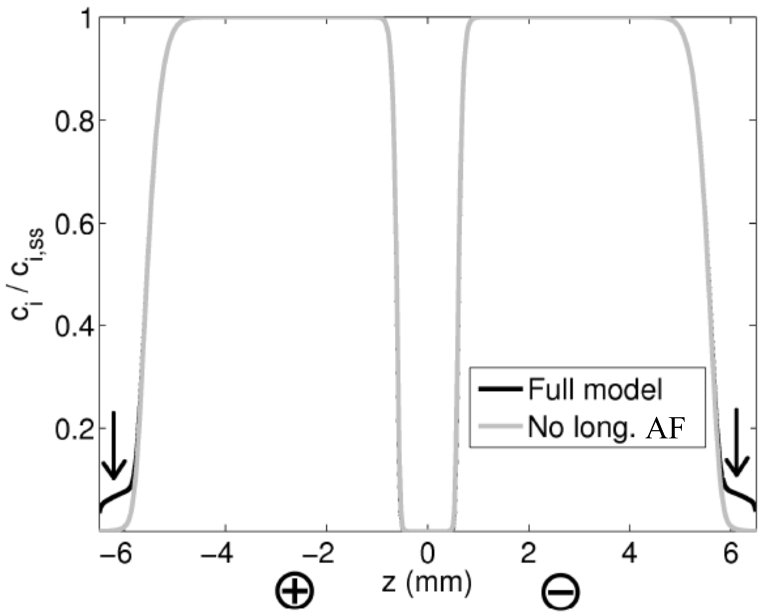
## Far Fiber




Longitudinal charging: 2000 %

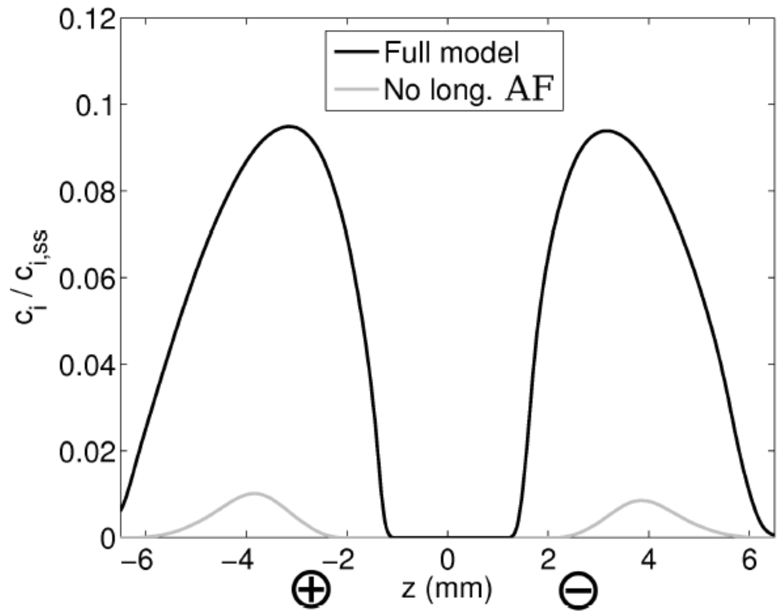
# Effect of Longitudinal Charging

Close Fiber

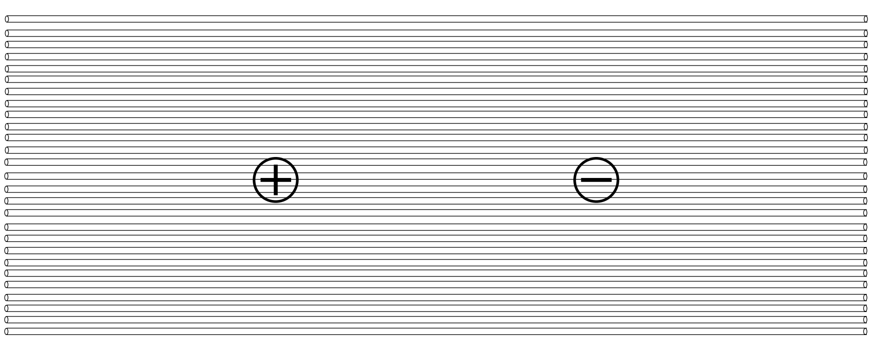



Longitudinal charging: 0.91 %

Far Fiber




Longitudinal charging: 2000 %

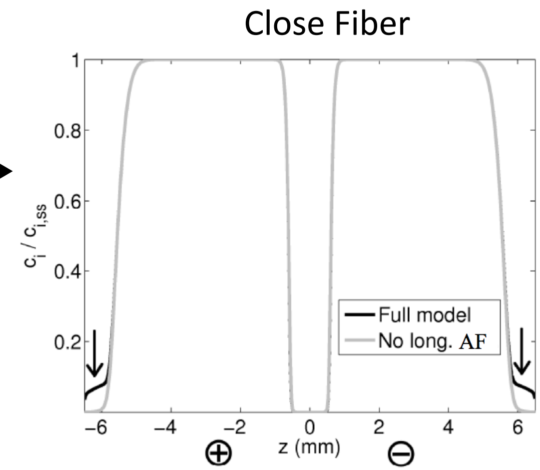


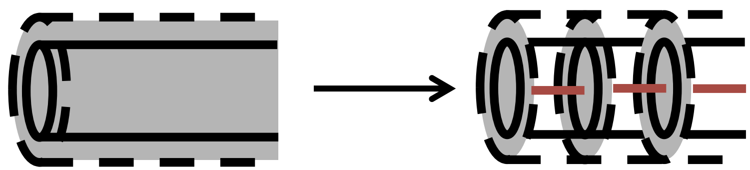

**Total uptake over all cells in tissue is increased by only 3.95% by including longitudinal AF, which is less than experimental variability (6%)**

# Versatility of Asymptotic Fiber Model

Neuron modeling community interested in stimulation of fibers

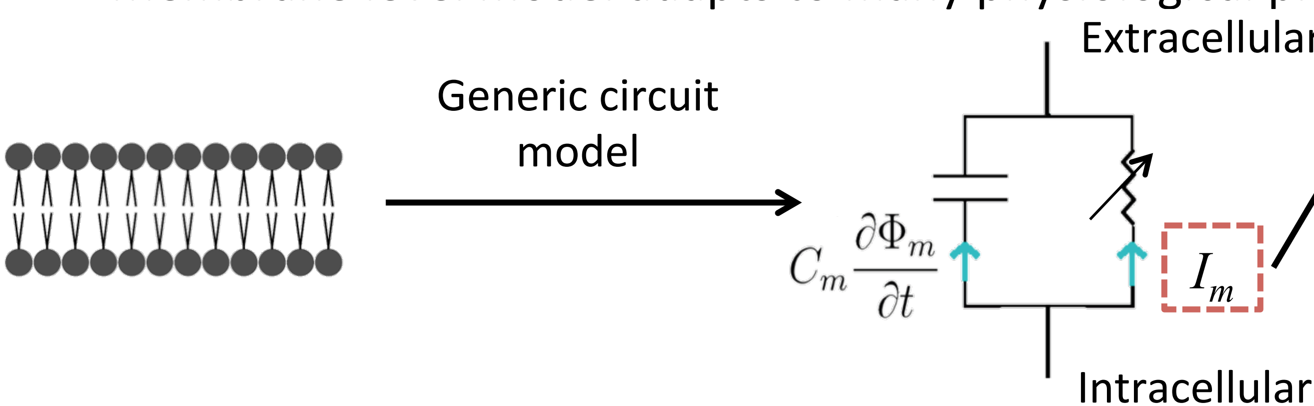
- Emphasis on longitudinal membrane charging (1D core conductor model), but …
- Longitudinal charging not important important for fibers close to electrodes
- Computationally efficient model to study transverse, as well as longitudinal charging



Model is valid for neurons

- Axon of neuron has similar geometry and electrical properties
- Membrane level model adapts to many physiological phenomena




<u>Adaptable</u>

- EP current for muscle EP
- AP current for neuron

# Conclusions

- Longitudinal charging dominates for skeletal muscle fibers far from electrodes, while transverse charging dominates for fibers close to the electrodes

- Majority of molecular uptake occurs for fibers close to the electrodes, thus, I corroborated traditional transverse-only models of electroporation in skeletal muscle

- Asymptotic model is an efficient and versatile tool to simulate bioelectric phenomena

- Presence of transverse charging in asymptotic model may have significant added value in simulating activation of neurons

# Acknowledgements

## Thank You

All Individuals in the Audience


Duke University Administrative and Technical Support

Kathy Barbour
Ned Danieley
Susan Story
Dr. Adam P. Wax
Duke OIT

Colleagues and Mentors

Dr. Roger C. Barr
Dr. Margot E. Bowen
Dr. James Esterline
Dr. Mazella B. Fuller
Dr. Thomas J. McIntosh
Dr. John C. Neu
**Dr. Wanda K. Neu**
Dr. Ann Pitruzzello
Dr. Caroline Ring
Dr. John Wambaugh
Dr. Thomas P. Witelski
Dr. Fan Yuan

# Extra Slides

# Experimental Studies and Modeling

Goal in published studies is to maximize gene expression

- Optimize pulsing protocol
- Electrode geometry

Modeling DNA and expression: complex

- Relate DNA uptake to expression
- DNA is long polymer
  - Stretches/shrinks
  - Endocytotic mechanisms
  - Membrane adsorption
  - Molecular dynamics (expensive)

Modeling small molecules: less complex

- Radiolabelled molecules
- Radioactivity → molecular concentration
- Molecular concentration: convection-diffusion equation (less expensive)

# Theoretical Studies

Membrane level

Creation of pores and current through pores in response to $\Phi_m$

Molecular dynamics model

- Motion of each lipid molecule
- Not suitable for spatially extended system

Asymptotic model of EP[1]

- Simple ODE
- Membrane of spherical cells

**Longitudinal behavior of EP in fibers not characterized**

*[1]Neu, J.C. & Krassowska, W. (1999) PRE, 59, 3471-3482*

# Theoretical Studies

<u>Cellular level</u>

Distribution of $\Phi_m$ due to microscale geometry of individual cells

EP community models

- $|\Phi_m| \propto |E|$

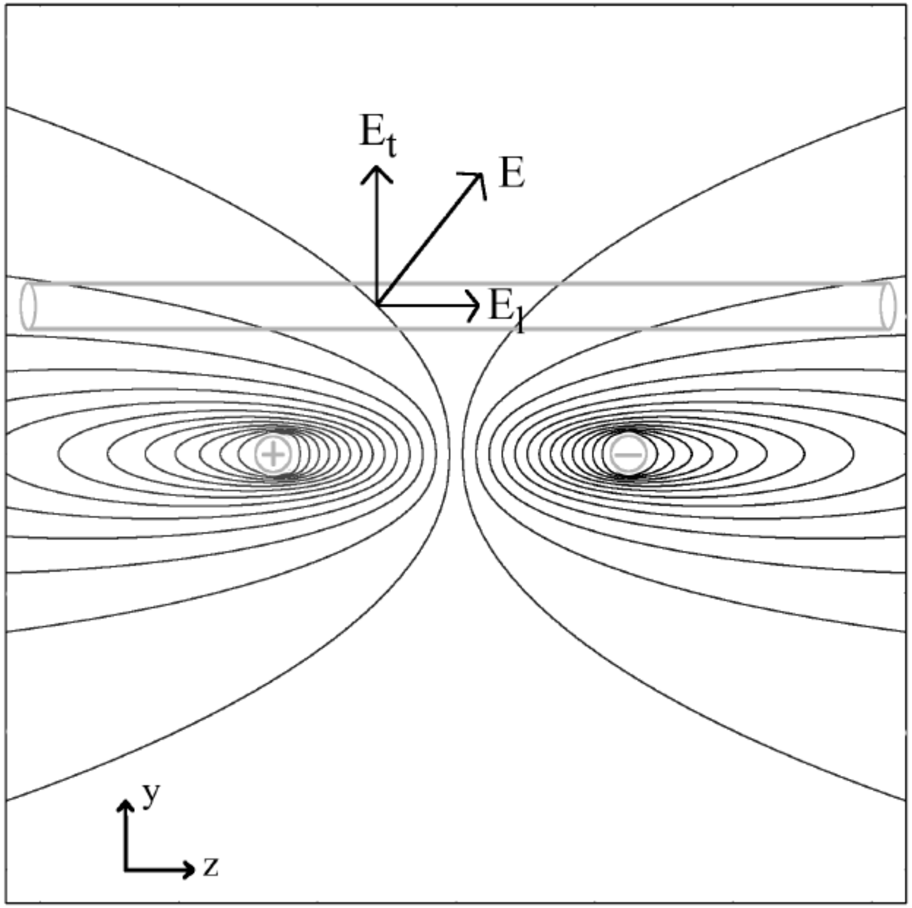
# Theoretical Studies

Cellular level

Distribution of $\Phi_m$ due to microscale geometry of individual cells

EP community models

- $|\Phi_m| \propto |E|$



# Theoretical Studies

## Cellular level

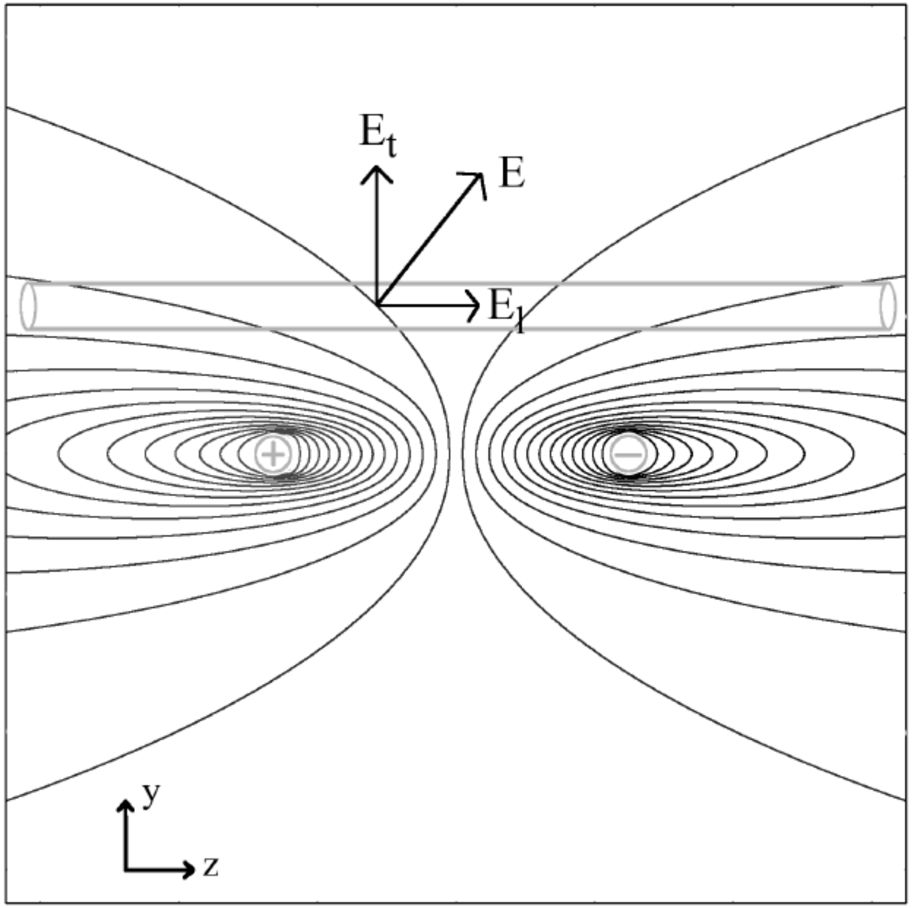
Distribution of $\Phi_m$ due to microscale geometry of individual cells

EP community models

- $|\Phi_m| \propto |E|$

Neuron community models

- "transverse charging"
  $|\Phi_m| \propto |E_t|$
- "longitudinal charging"
  $|\Phi_m| \propto \left|\frac{\partial E_l}{\partial z}\right|$
- $\left|\frac{\partial E_l}{\partial z}\right|$ varies by 7 orders of mag.




**Effect of longitudinal charging on EP not characterized**

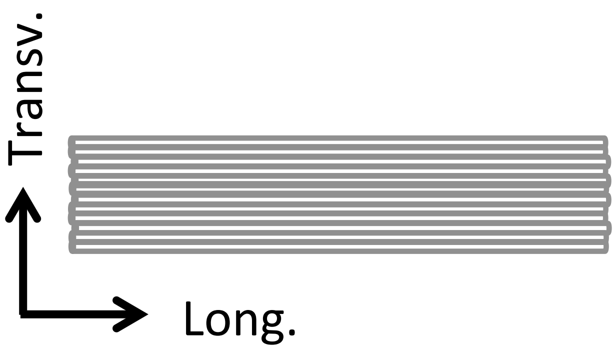
# Theoretical Studies

## Tissue level

Connects macroscale tissue distribution of potential from electric stimulus to $\Phi_m$ at **cellular level**

### Ideal tissue model

Individual fibers



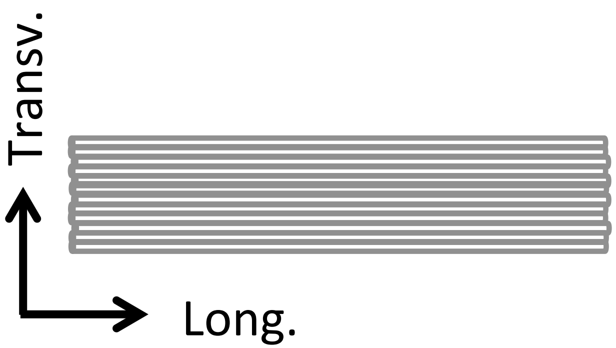
# Theoretical Studies

## Tissue level

Connects macroscale tissue distribution of potential from electric stimulus to $\Phi_m$ at **cellular level**

### Ideal tissue model

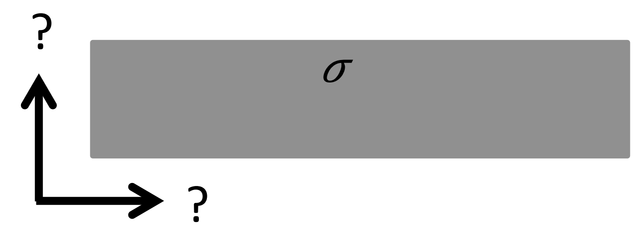
Individual fibers




### Existing models

No individual fiber geometry

Vs.




**Existing models cannot capture transverse and longitudinal membrane charging**

# Research Aims

1. Investigate mechanism of transverse and longitudinal membrane charging during EP

2. Determine contribution of longitudinal charging to molecular uptake during EP

3. Compare uptake to traditional models of EP in muscle that neglect individual fiber geometry, and thus, directional membrane charging

# Outline

## Introduction

- Background
- Published experiments and models

## **Derivation and Validation of Models**

- **Asymptotic fiber model**
- Alterations for EP-mediated uptake
- Mass transport model

## Results

- Single fiber close to electrodes
- Single fiber far from electrodes
- Model variations and tissue-wide effect

## Conclusions and Connections to LLNL

# Theoretical Studies

<u>Tissue level</u>

- Macroscale distribution of potential throughout entire tissue
- Connects electric stimulus to membrane charging at cellular level

Liver tissue models

- Compact, spherical cells
- Asymptotic model of EP

Muscle tissue models

- No individual cell geometry
- Cannot capture transverse and longitudinal membrane charging

**Effect of individual fiber geometry on EP in muscle tissue not characterized**

# Theoretical Studies

## Mass transport

Movement of small molecules throughout tissue and across membrane

Transport mechanisms

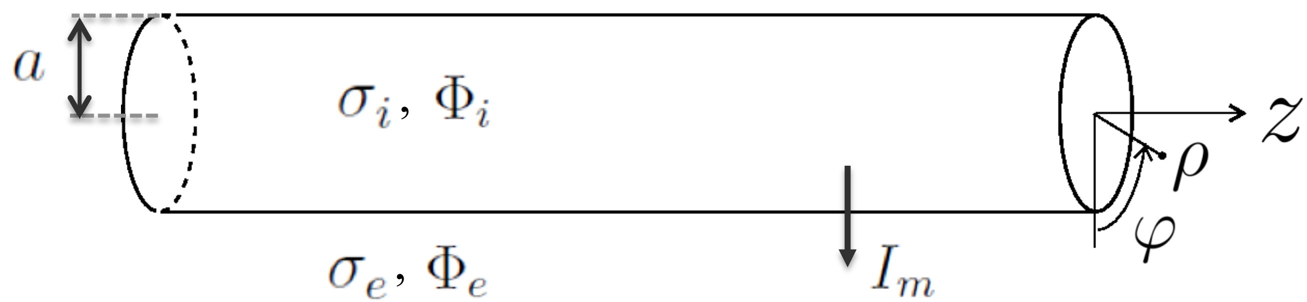
- Electric drift
- Diffusion
- Convection-diffusion equation

Applied to cancer tissue

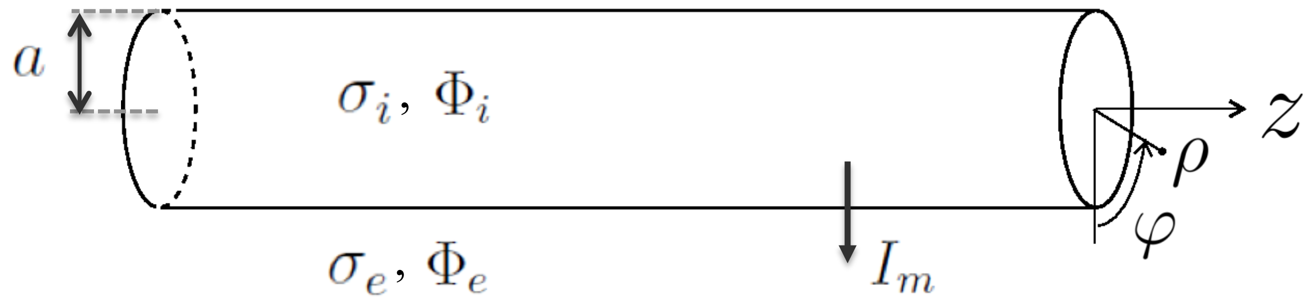
- Compact spherical cells

**Uptake in muscle tissue with longitudinal and transverse membrane charging not characterized**

# Derivation of Asymptotic Fiber Model



# Derivation of Asymptotic Fiber Model




Scaled governing equations in dimensionless form

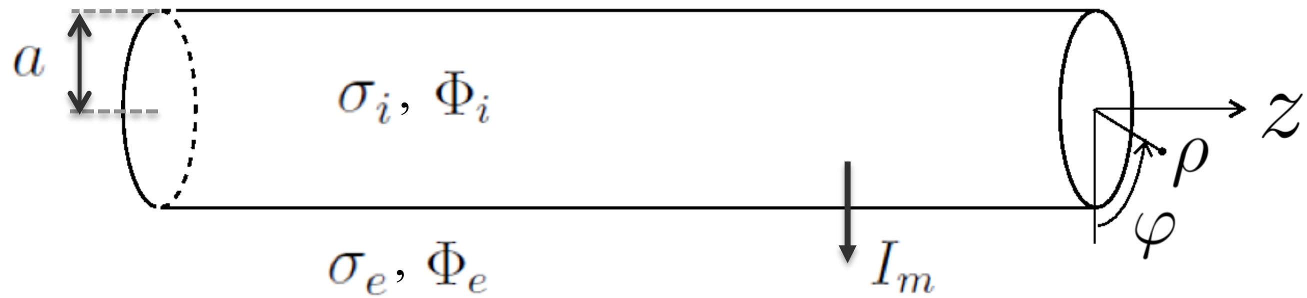
$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho\frac{\partial \Phi_i}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \Phi_i}{\partial \varphi^2} + \epsilon\frac{\partial^2 \Phi_i}{\partial z^2} = 0 \qquad \text{in } \rho < a,$$

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho\frac{\partial \Phi_e}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \Phi_e}{\partial \varphi^2} + \epsilon\frac{\partial^2 \Phi_e}{\partial z^2} = 0 \qquad \text{in } \rho > a,$$

$$\frac{\partial \Phi_i}{\partial \rho} = -\frac{\partial \Phi_m}{\partial \tau} - \epsilon\frac{\partial \Phi_m}{\partial T_s} - I_m - \frac{\partial \Psi}{\partial \rho} \qquad \text{on } \rho = a$$

$$\frac{\partial \Phi_e}{\partial \rho} = -\frac{\sigma_i}{\sigma_e}\left\{\frac{\partial \Phi_m}{\partial \tau} + \epsilon\frac{\partial \Phi_m}{\partial T_s} + I_m\right\} - \frac{\partial \Psi}{\partial \rho} \qquad \text{on } \rho = a$$

# Derivation of Asymptotic Fiber Model




Scaled governing equations in dimensionless form

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho \frac{\partial \Phi_i}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \Phi_i}{\partial \varphi^2} + \boxed{\epsilon \frac{\partial^2 \Phi_i}{\partial z^2}} = 0 \qquad \text{in } \rho < a,$$

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho \frac{\partial \Phi_e}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \Phi_e}{\partial \varphi^2} + \boxed{\epsilon \frac{\partial^2 \Phi_e}{\partial z^2}} = 0 \qquad \text{in } \rho > a,$$

$$\frac{\partial \Phi_i}{\partial \rho} = -\frac{\partial \Phi_m}{\partial \tau} - \boxed{\epsilon \frac{\partial \Phi_m}{\partial T_s}} - I_m - \frac{\partial \Psi}{\partial \rho} \qquad \text{on } \rho = a$$

$$\frac{\partial \Phi_e}{\partial \rho} = -\frac{\sigma_i}{\sigma_e}\left\{\frac{\partial \Phi_m}{\partial \tau} + \boxed{\epsilon \frac{\partial \Phi_m}{\partial T_s}} + I_m\right\} - \frac{\partial \Psi}{\partial \rho} \qquad \text{on } \rho = a$$

# Derivation of Asymptotic Fiber Model

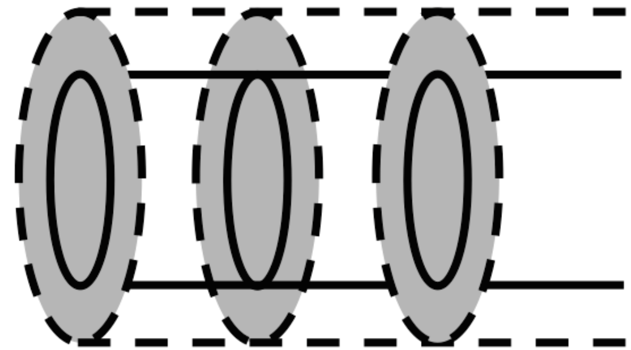
Mean-free equations of the transverse problem

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_i^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_i^0}{\partial\varphi^2}=0 \qquad \text{in } \rho<a$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_e^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_e^0}{\partial\varphi^2}=0 \qquad \text{in } \rho>a$$

$$\sigma_i\frac{\partial\phi_i^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_i\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$

$$\sigma_e\frac{\partial\phi_e^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_e\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$



# Derivation of Asymptotic Fiber Model

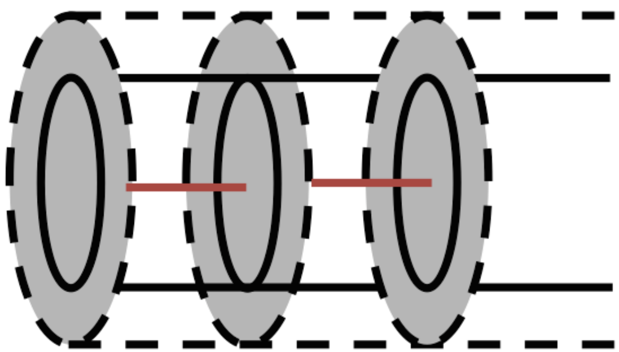
Mean-free equations of the transverse problem

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_i^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_i^0}{\partial\varphi^2}=0 \qquad \text{in } \rho<a$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_e^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_e^0}{\partial\varphi^2}=0 \qquad \text{in } \rho>a$$

$$\sigma_i\frac{\partial\phi_i^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_i\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$

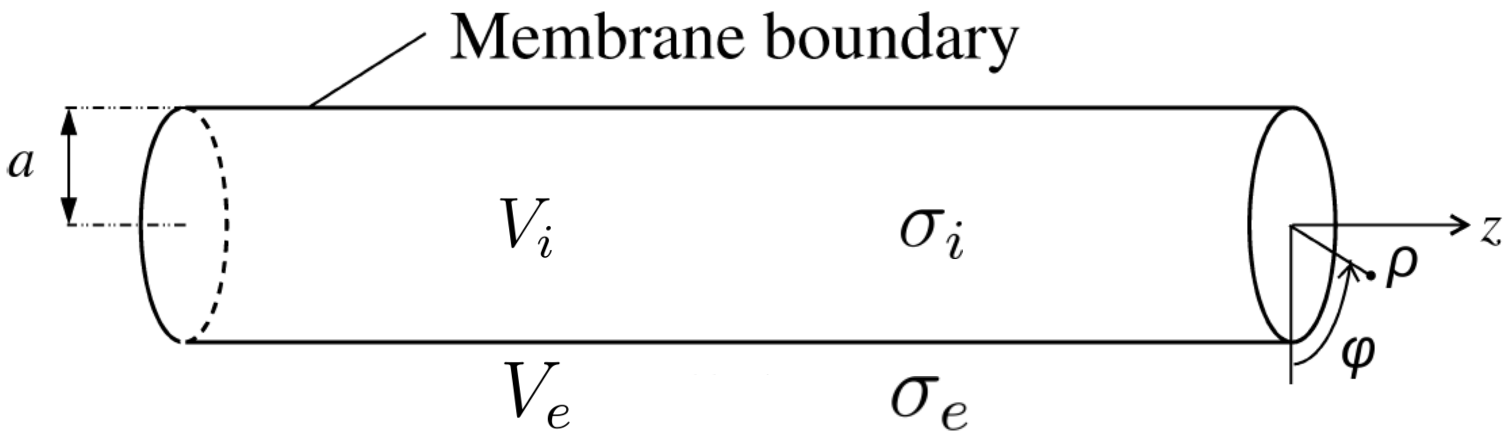
$$\sigma_e\frac{\partial\phi_e^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_e\frac{\partial\widetilde{\Psi}}{\partial\rho} \qquad \text{on } \rho=a$$



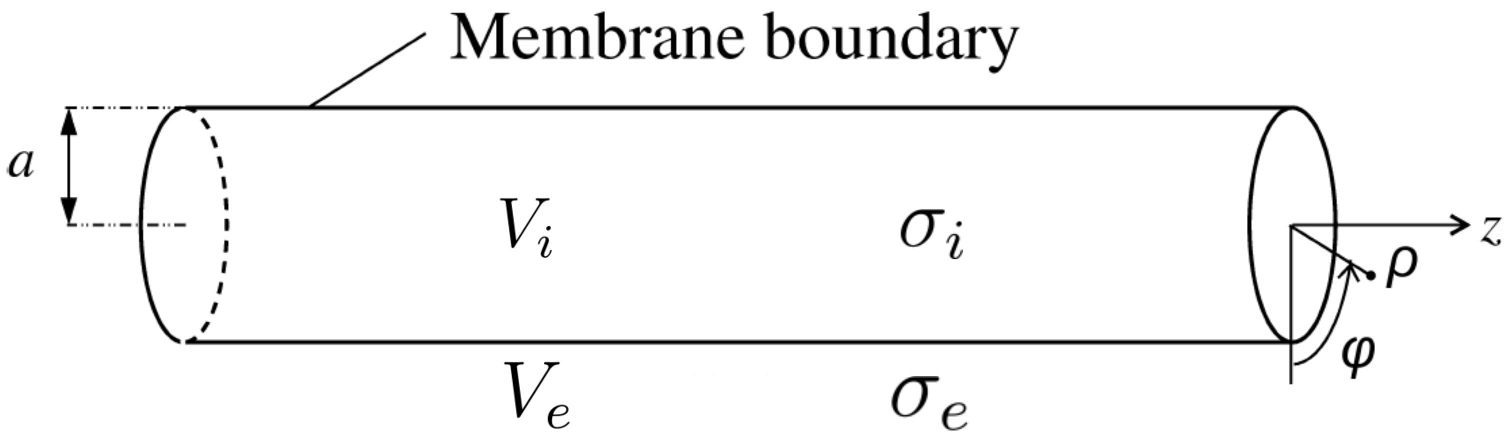
Mean equation of the longitudinal problem

$$\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}=C_m\frac{\partial f_m^0}{\partial t}+\overline{I_m}-\frac{\sigma_i a}{2}\frac{\partial^2\left\langle\Psi\right\rangle}{\partial z^2}$$

# Derivation of Asymptotic Fiber Model



# Derivation of Asymptotic Fiber Model




Split potential

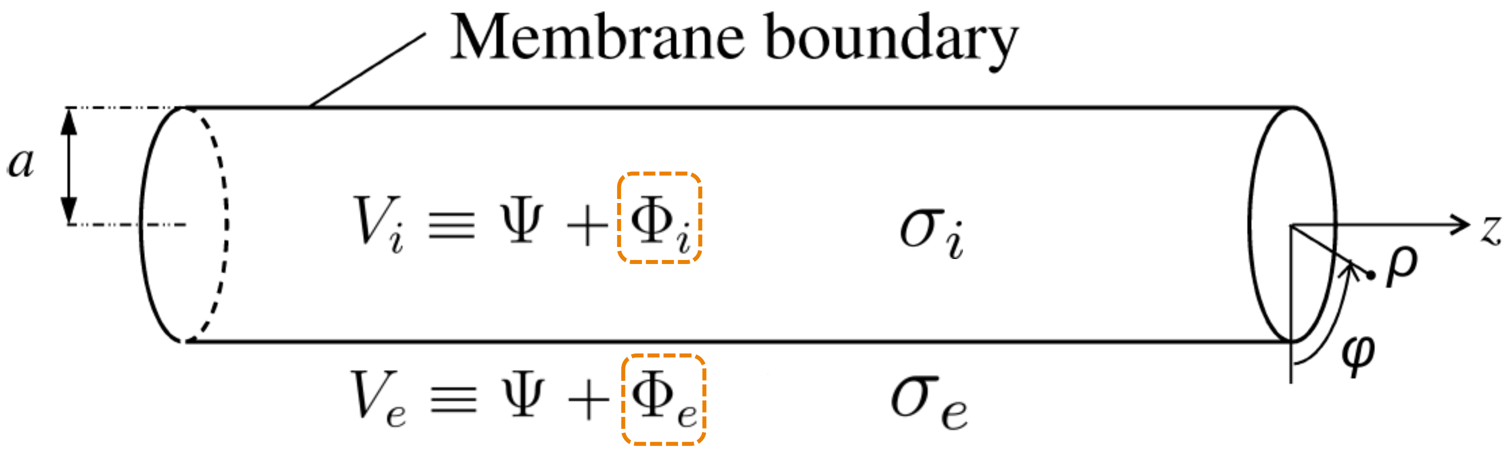
$$V_i \equiv \boxed{\Psi} + \Phi_i$$

$$V_e \equiv \boxed{\Psi} + \Phi_e$$

Primary potential $\boxed{\Psi}$

- Excludes microscopic presence of fibers
- Analytical solution

# Derivation of Asymptotic Fiber Model




Secondary potentials $\boxed{\Phi_i}$ and $\boxed{\Phi_e}$

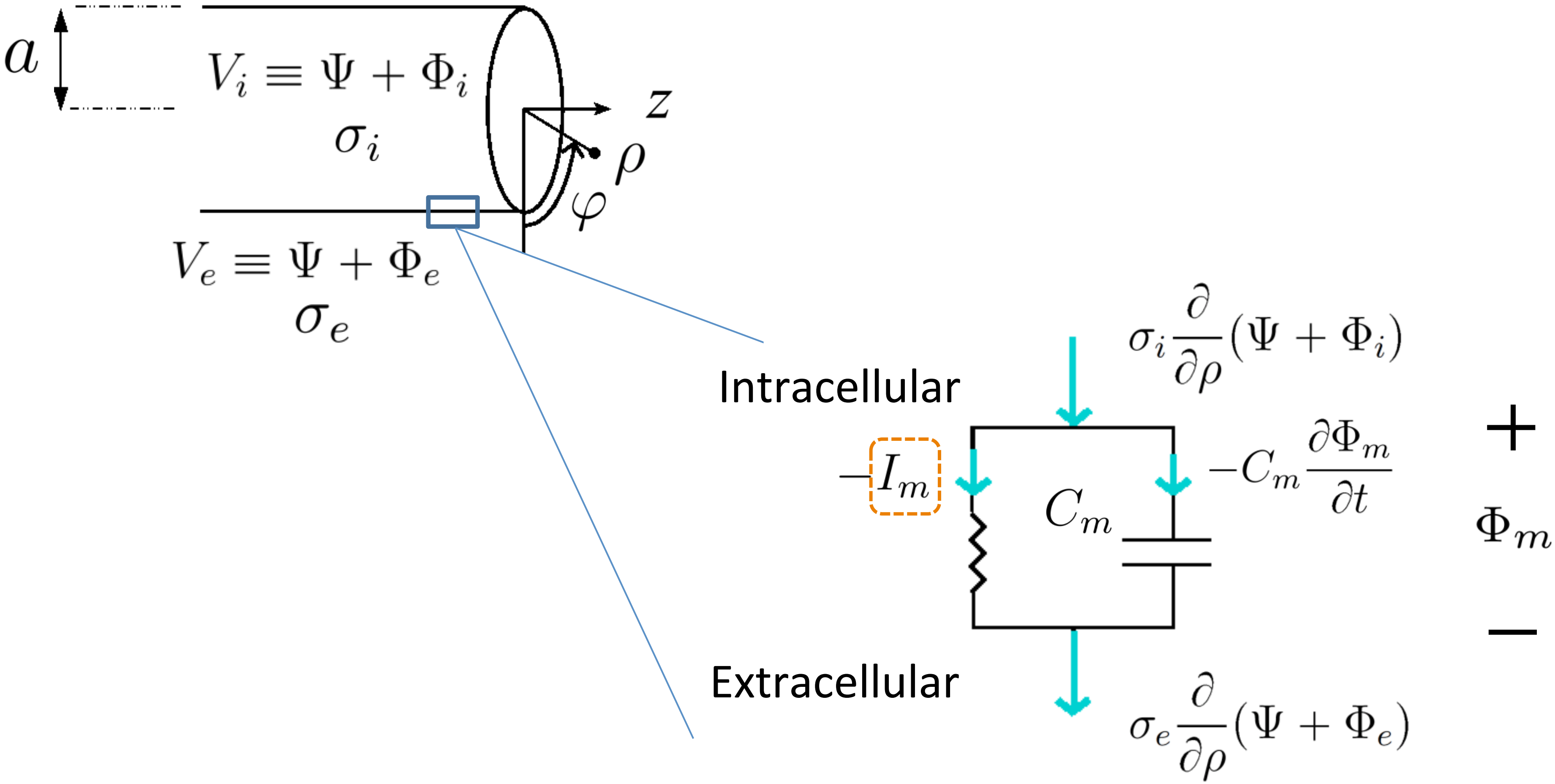
- Includes microscopic presence of fiber

- Laplace's equation

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\Phi_i}{\partial\rho}\right) + \frac{1}{\rho^2}\frac{\partial^2\Phi_i}{\partial\varphi^2} + \frac{\partial^2\Phi_i}{\partial z^2} = 0 \qquad \text{in } \rho < a$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\Phi_e}{\partial\rho}\right) + \frac{1}{\rho^2}\frac{\partial^2\Phi_e}{\partial\varphi^2} + \frac{\partial^2\Phi_e}{\partial z^2} = 0 \qquad \text{in } \rho > a$$

# Derivation of Asymptotic Fiber Model




- Matching conditions

$$\sigma_i \frac{\partial}{\partial \rho}(\Psi + \Phi_i) = \sigma_e \frac{\partial}{\partial \rho}(\Psi + \Phi_e) = -C_m \frac{\partial \Phi_m}{\partial t} - \boxed{I_m} \quad \text{on } \rho = a$$

# Derivation of Asymptotic Fiber Model

Spatial scales

Short: microns (fiber radius)

Long: millimeters (length scale of fiber)

Temporal scales

Fast: microseconds (charging by transverse currents, $aC_m/\sigma_i$ )

Slow: milliseconds (charging by longitudinal currents, $R_mC_m$)

Separate *fast, short*-distance response from *slow, long*-distance response

Method of multiple scales $\dfrac{\partial}{\partial t} = \dfrac{\partial}{\partial \tau} + \epsilon \dfrac{\partial}{\partial T_s}$

Small parameter: $\epsilon = \dfrac{aC_m/\sigma_i}{R_mC_m} = \dfrac{a}{\sigma_i R_m}$ → $O(10^{-5})$

# Derivation of Asymptotic Fiber Model

Scale and convert governing equations to dimensionless form

Expand potentials in powers of $\epsilon$ , e.g., $\Phi_i \sim \Phi_i^0 + \epsilon \Phi_i^1 + \cdots$

Limit $\epsilon \rightarrow 0$, LO problem suggests separation of potentials

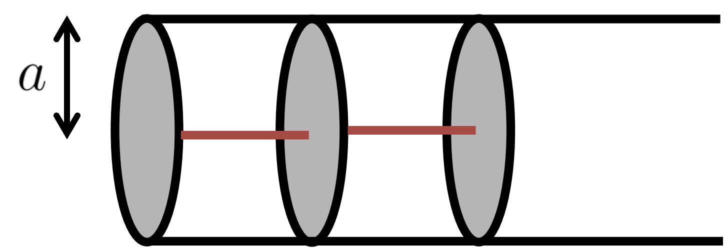
$$\Phi_i^0 = \phi_i^0(\rho, \varphi, \tau) + f_i^0(z, T_s)$$

$$\Phi_e^0 = \phi_e^0(\rho, \varphi, \tau) + f_e^0(z, T_s)$$

$$\Phi_m^0 = \underbrace{\phi_m^0(\varphi, \tau)}_{mean-free} + \underbrace{f_m^0(z, T_s)}_{mean}$$

Definition of mean and mean-free components

$$\underbrace{\phi_i^0}_{mean-free} = \underbrace{\Phi_i^0}_{total} - \underbrace{\frac{1}{\pi a^2} \int_0^{2\pi} \int_0^a \Phi_i^0 \; \rho d\rho d\varphi}_{mean}$$



# Derivation of Asymptotic Fiber Model

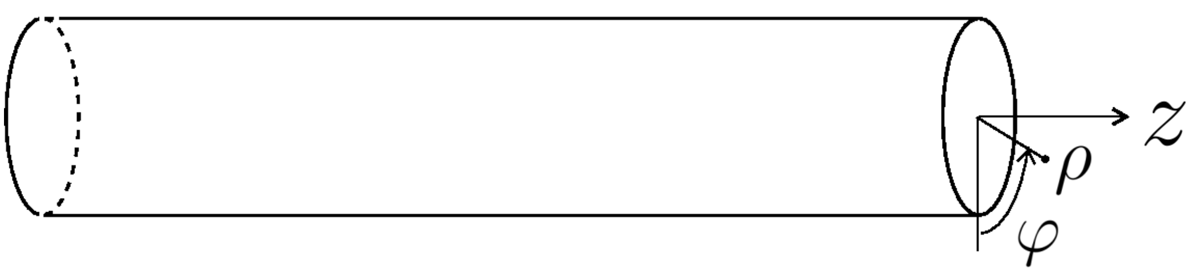
Scale and convert governing equations to dimensionless form

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\Phi_i}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\Phi_i}{\partial\varphi^2}+\cancel{\epsilon\frac{\partial^2\Phi_i}{\partial z^2}}=0 \qquad \text{in } \rho<a$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\Phi_e}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\Phi_e}{\partial\varphi^2}+\cancel{\epsilon\frac{\partial^2\Phi_e}{\partial z^2}}=0 \qquad \text{in } \rho>a$$

$$\frac{\partial\Phi_i}{\partial\rho}=-\frac{\partial\Phi_m}{\partial\tau}-\cancel{\epsilon\frac{\partial\Phi_m}{\partial T_s}}-I_m-\frac{\partial\Psi}{\partial\rho} \qquad \text{on } \rho=a$$

$$\frac{\partial\Phi_e}{\partial\rho}=-\frac{\sigma_i}{\sigma_e}\left\{\frac{\partial\Phi_m}{\partial\tau}+\cancel{\epsilon\frac{\partial\Phi_m}{\partial T_s}}+I_m\right\}-\frac{\partial\Psi}{\partial\rho} \qquad \text{on } \rho=a$$



# Derivation of Asymptotic Fiber Model

Limit $\epsilon \to 0$, LO problem suggests separation of potentials

$$\Phi_i^0 = \phi_i^0(\rho, \varphi, \tau) + f_i^0(z, T_s)$$

$$\Phi_e^0 = \phi_e^0(\rho, \varphi, \tau) + f_e^0(z, T_s)$$

$$\Phi_m^0 = \underbrace{\phi_m^0(\varphi, \tau)}_{mean-free} + \underbrace{f_m^0(z, T_s)}_{mean}$$

# Derivation of Asymptotic Fiber Model

Limit $\epsilon \rightarrow 0$, LO problem suggests separation of potentials

$$\Phi_i^0 = \phi_i^0(\rho, \varphi, \tau) + f_i^0(z, T_s)$$

$$\Phi_e^0 = \phi_e^0(\rho, \varphi, \tau) + f_e^0(z, T_s)$$

$$\Phi_m^0 = \underbrace{\phi_m^0(\varphi, \tau)}_{mean-free} + \underbrace{f_m^0(z, T_s)}_{mean}$$

Mean-free equations of the transverse problem

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho \frac{\partial \phi_i^0}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \phi_i^0}{\partial \varphi^2} = 0 \qquad \text{in } \rho < a,$$

$$\frac{1}{\rho}\frac{\partial}{\partial \rho}\left(\rho \frac{\partial \phi_e^0}{\partial \rho}\right) + \frac{1}{\rho^2}\frac{\partial^2 \phi_e^0}{\partial \varphi^2} = 0 \qquad \text{in } \rho > a,$$

$$\sigma_i \frac{\partial \phi_i^0}{\partial \rho} = -C_m \frac{\partial \phi_m^0}{\partial t} - \widetilde{I_m} - \sigma_i \frac{\partial \widetilde{\Psi}}{\partial \rho} \qquad \text{on } \rho = a$$

$$\sigma_e \frac{\partial \phi_e^0}{\partial \rho} = -C_m \frac{\partial \phi_m^0}{\partial t} - \widetilde{I_m} - \sigma_e \frac{\partial \widetilde{\Psi}}{\partial \rho} \qquad \text{on } \rho = a$$

# Derivation of Asymptotic Fiber Model

## Mean-free equations of the transverse problem

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_i^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_i^0}{\partial\varphi^2}=0 \qquad \text{in } \rho<a,$$

$$\frac{1}{\rho}\frac{\partial}{\partial\rho}\left(\rho\frac{\partial\phi_e^0}{\partial\rho}\right)+\frac{1}{\rho^2}\frac{\partial^2\phi_e^0}{\partial\varphi^2}=0 \qquad \text{in } \rho>a,$$

$$\sigma_i\frac{\partial\phi_i^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_i\boxed{\frac{\partial\widetilde{\Psi}}{\partial\rho}} \qquad \text{on } \rho=a$$

$$\sigma_e\frac{\partial\phi_e^0}{\partial\rho}=-C_m\frac{\partial\phi_m^0}{\partial t}-\widetilde{I_m}-\sigma_e\boxed{\frac{\partial\widetilde{\Psi}}{\partial\rho}} \qquad \text{on } \rho=a$$

$\partial\widetilde{\Psi}/\partial\rho$ : "transverse AF"

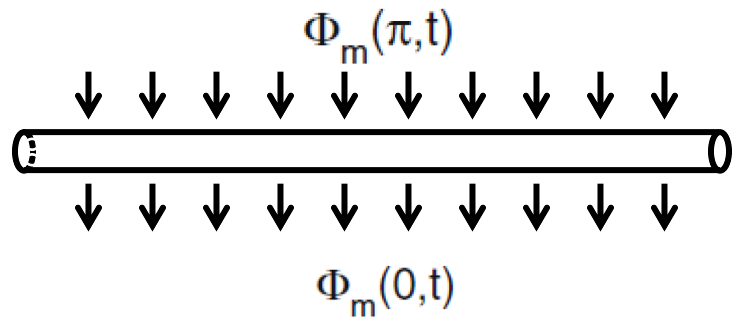
## Mean equation of the longitudinal problem

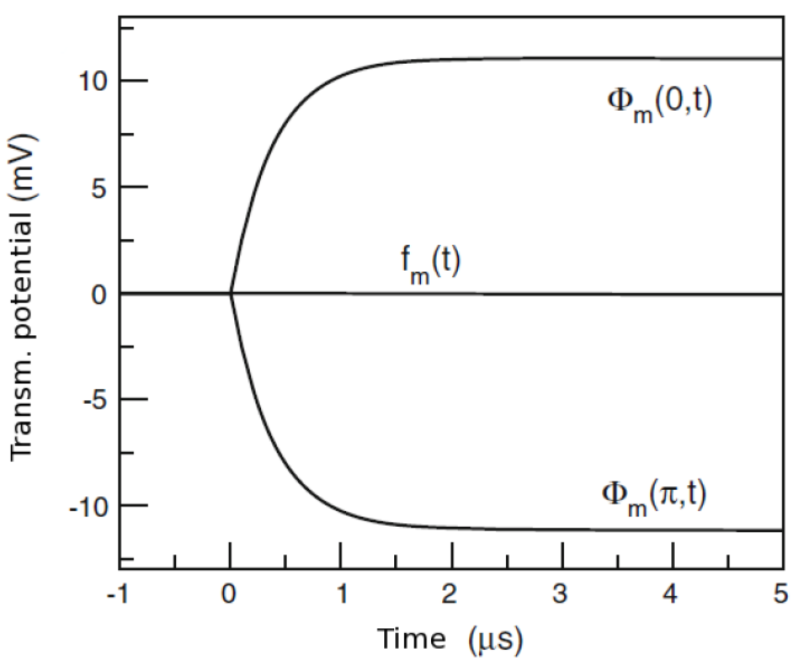
$$\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}=C_m\frac{\partial f_m^0}{\partial t}+\overline{I_m}-\frac{\sigma_i a}{2}\boxed{\frac{\partial^2\langle\Psi\rangle}{\partial z^2}}$$

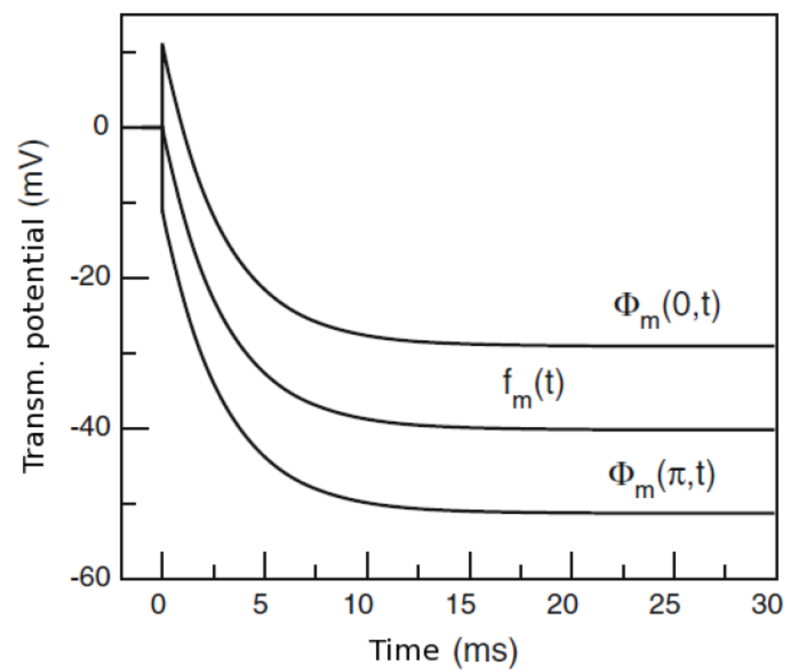
$\dfrac{\partial^2\langle\Psi\rangle}{\partial z^2}$ : "longitudinal AF"

# Validation of Asymptotic Fiber Model

## Validation of temporal scales separation

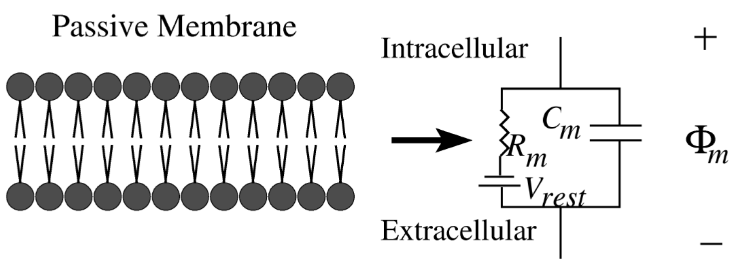


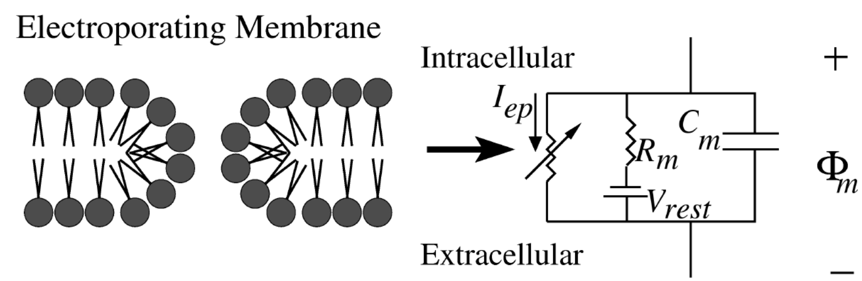



# Alterations for EP-Mediated Uptake

## Addition of current due to EP




$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m} + I_{ep}$$

$$I_{ep} = \boxed{N_{ep}} i_{ep}$$

### Pore density

Model: Asymptotic model of EP[1]

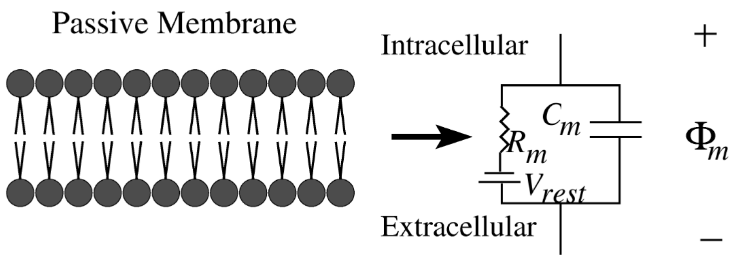
Assumptions

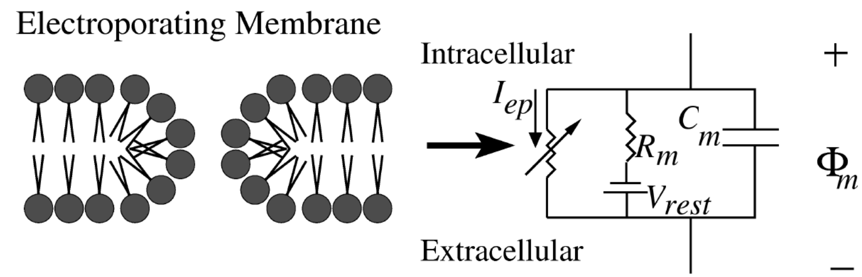
- Non-growing, constant pore radius
- Parameters fit to artificial membrane

[1]*Neu, J.C. & Krassowska, W. (1999) PRE, 59, 3471-3482*

# Alterations for EP-Mediated Uptake

## Addition of current due to EP




$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m} + I_{ep}$$

$$I_{ep} = N_{ep}\boxed{i_{ep}}$$

### Pore density

Model: Asymptotic model of EP[1]

Assumptions

- Non-growing, constant pore radius
- Parameters fit to artificial membrane

### Current through an electropore

Model: Aqueous pore[2]

Assumptions

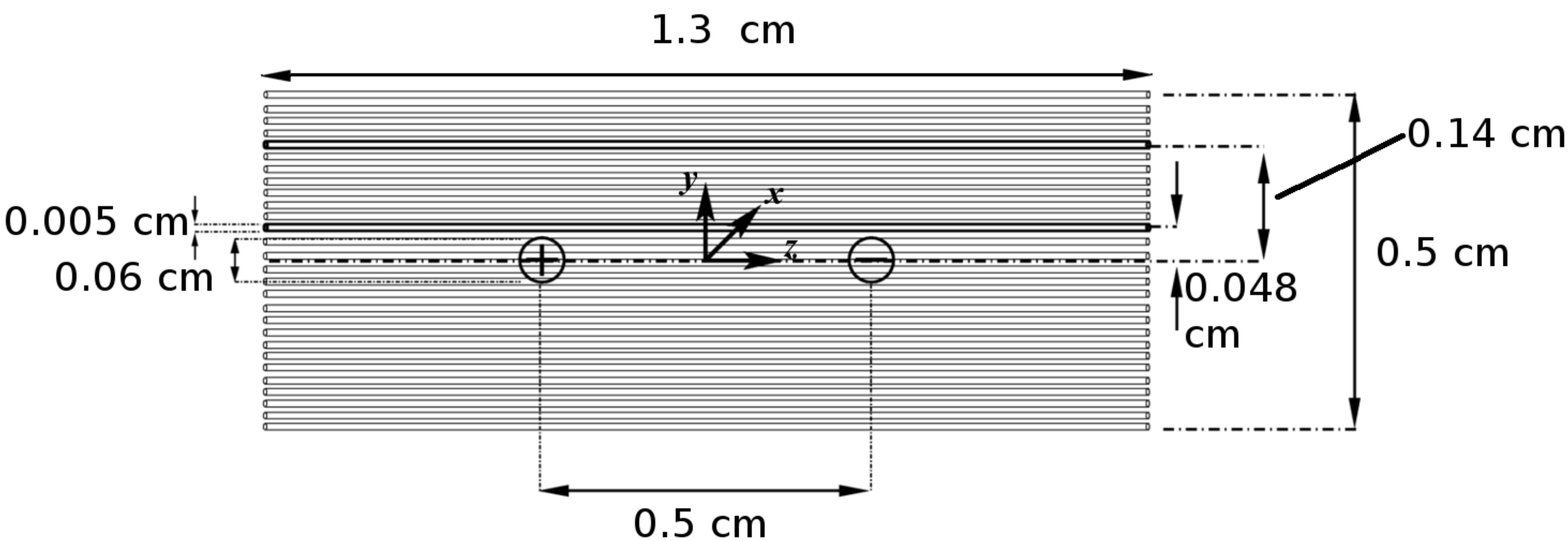
- Born energy due to low permittivity
- Two singularly charged ions (+ and -)
- Parameters fit to artificial membrane

[1] *Neu, J.C. & Krassowska, W. (1999) PRE, 59, 3471-3482*

[2] *Barnett, A. (1999) Biochim. Biophys. Acta, 1025, 10-14*

# Alterations for EP-Mediated Uptake

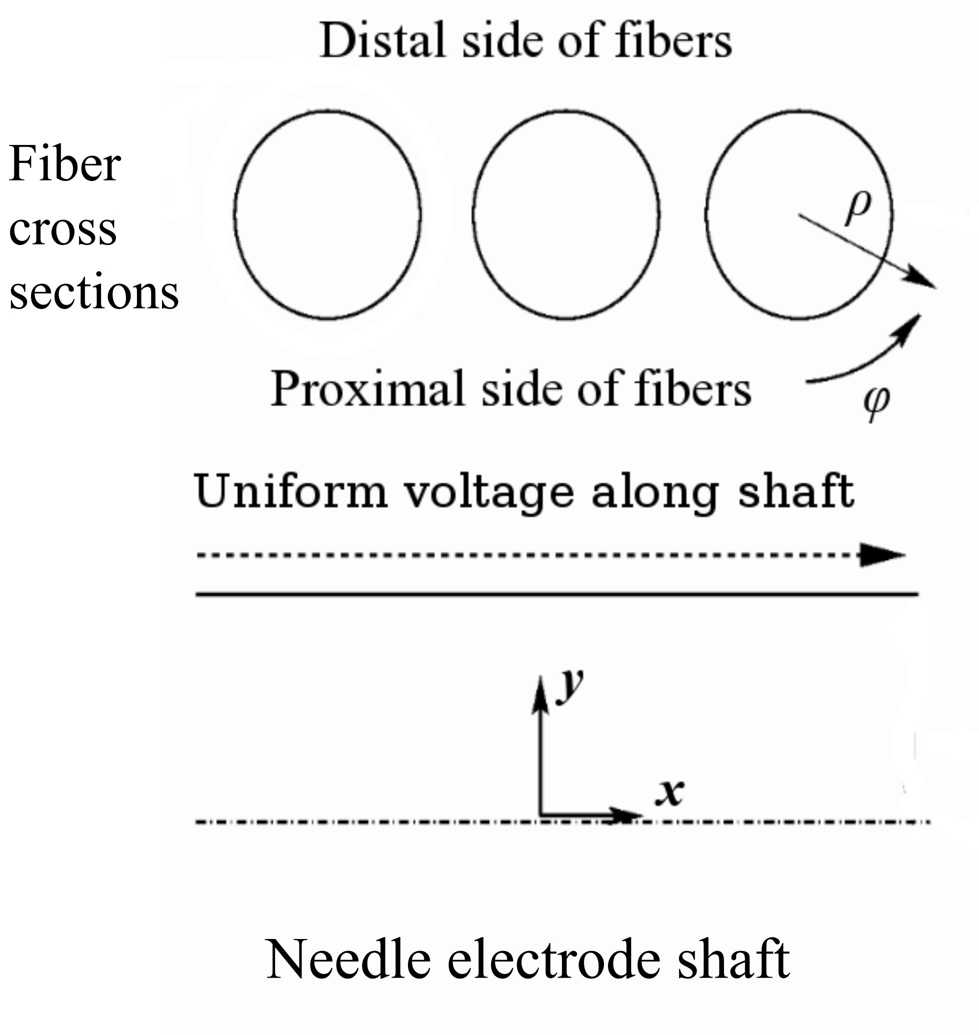
## Conversion to muscle tissue




- Geometry from experiments
- Interfiber distance 1/50 fiber diameter
- Needle diameter is 12x fiber diameter
- Fiber length is 260x fiber diameter
- Far fiber is 3x distance of close fiber

# Alterations for EP-Mediated Uptake

## Conversion to muscle tissue




Fibers identical in $x$-dimension

Simulate fibers at different distances in $y$-dimension

- Each fiber has different AFs $\partial\widetilde{\Psi}/\partial\rho$ and $\partial^2 \langle\Psi\rangle/\partial z^2$
- Analytical solution to primary potential $\Psi$ in anisotropic medium[1]

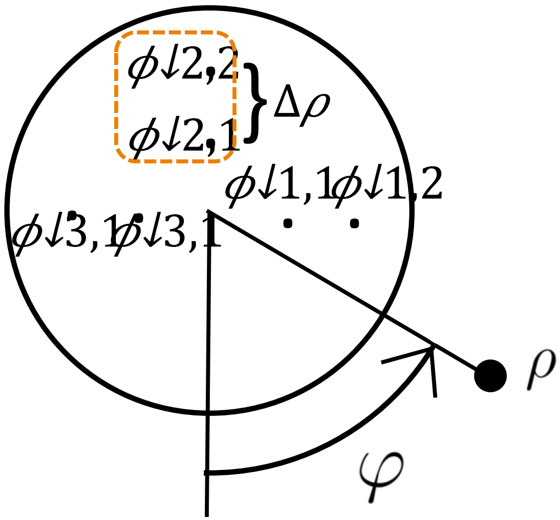
$$\sigma_y \frac{\partial^2\Psi}{\partial y^2} + \sigma_z \frac{\partial^2\Psi}{\partial z^2} = 0$$

[1] *Guo, L., Cranford, J.P., Neu, J.C., and Neu, W.K. (2009) Med. Biol. Eng. Comput., 47, 1001-1010*

# Numerical Implementation and Simulation

**Transverse 2D problem**

Spatial: Finite difference method (FDM)

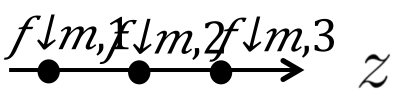



Discretization: $\frac{\partial \phi}{\partial \rho} \rightarrow \frac{\phi_{2,2} - \phi_{2,1}}{\Delta \rho}$

Temporal: Backward difference

Discretization: $\frac{\partial \phi}{\partial t} = \mathbb{F} \rightarrow \frac{\phi^{t+\Delta t} - \phi^{t}}{\Delta t} = \mathbb{F}^{t+\Delta t}$

**Longitudinal 1D problem: Crank-Nicolson method**



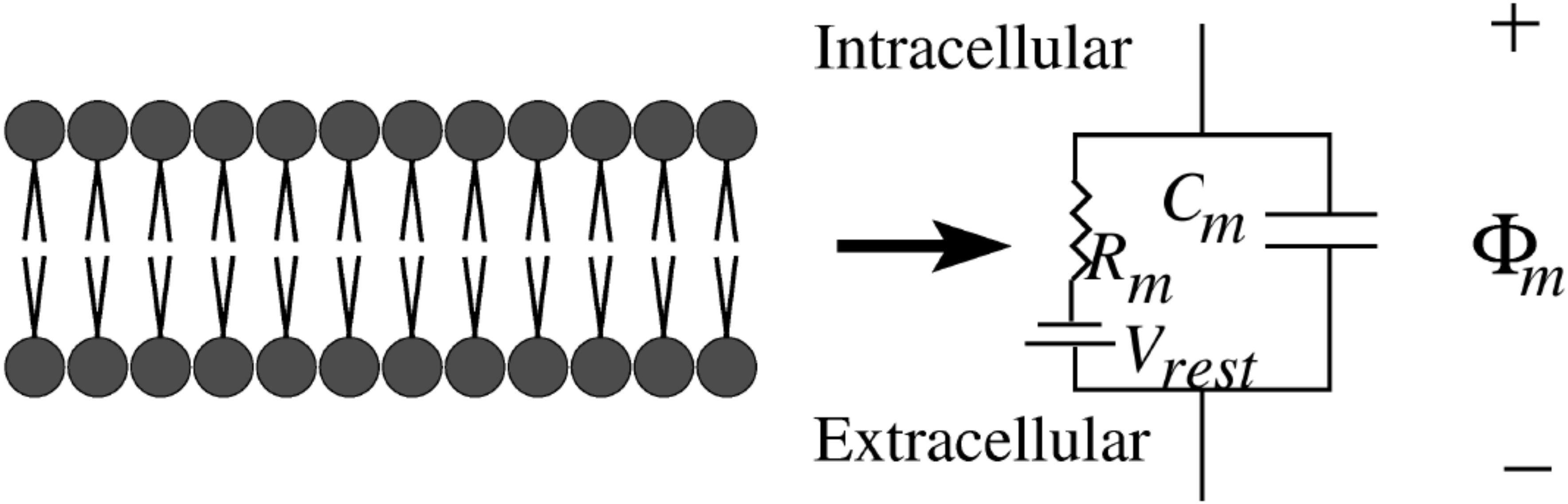

Discretization: $\frac{\partial f_m}{\partial t} = \mathbb{F} \rightarrow \frac{f_m^{t+\Delta t} - f_m^{t}}{\Delta t} = \frac{1}{2}\left\{\mathbb{F}^{t+\Delta t} + \mathbb{F}^{t}\right\}$

**Parallel computing?**

System in "anti-Goldilocks zone"

# Validation of Asymptotic Fiber Model

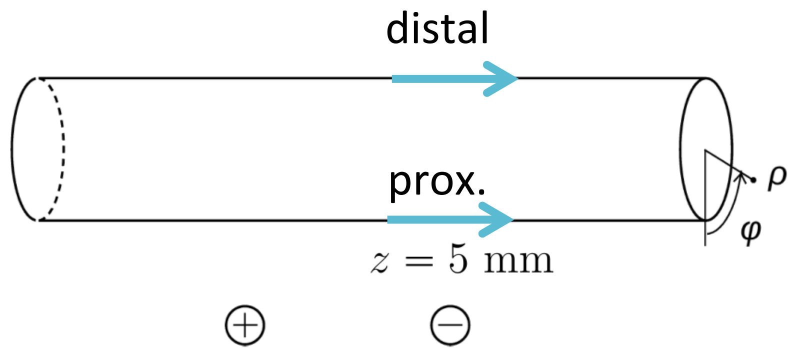
## Passive membrane dynamics



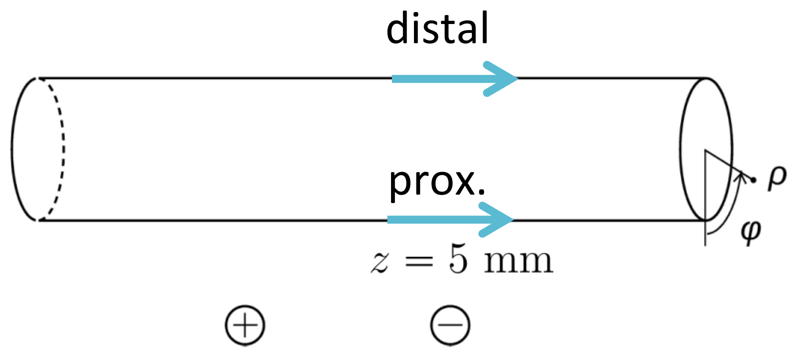

Q: Why passive (no pores/action potentials in membrane)?

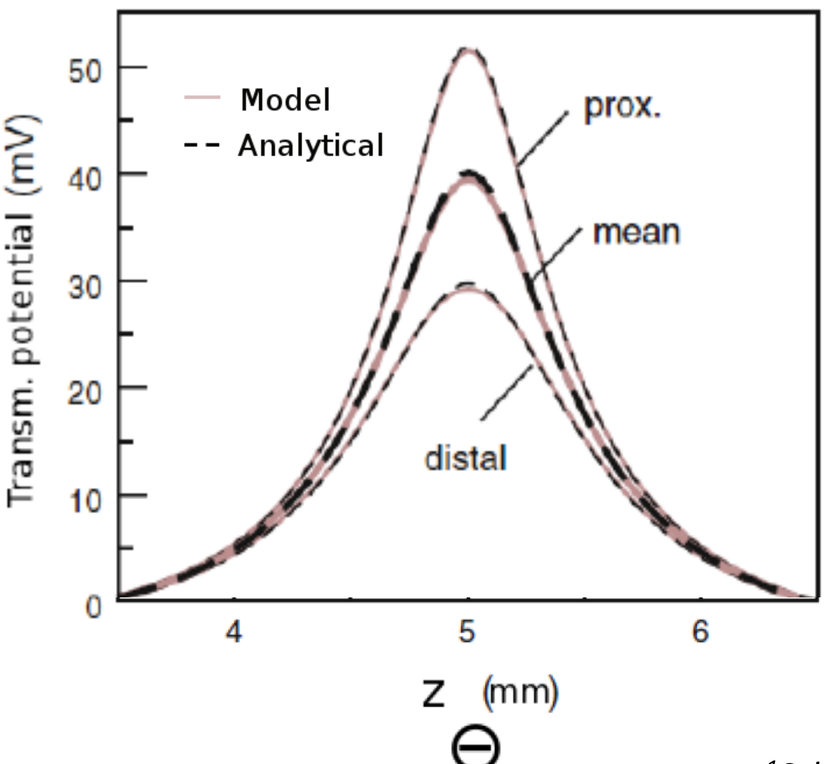
A: So there is an analytical solution!

# Validation of Asymptotic Fiber Model



# Validation of Asymptotic Fiber Model




Compare distribution of steady state $\Phi_m$ in model to analytical solution[1]

Root mean square error: 0.153 mV (0.295% of max $|\Phi_m|$)

[1]*Schnabel, V. and Struijk, J.J. (2001) IEEE T. Bio-Med. Eng., 48, 1027-1033*

# Outline

Introduction

- Background
- Published experiments and models

**Derivation and Validation of Models**

- Asymptotic fiber model
- **Alterations for EP-mediated uptake**
- Mass transport model

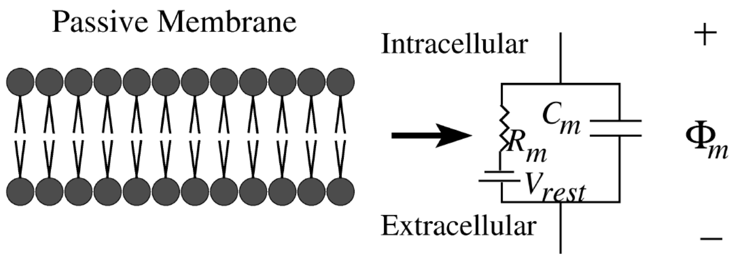
Results

- Single fiber close to electrodes
- Single fiber far from electrodes
- Model variations and tissue-wide effect

Conclusions and Connections to LLNL

# Alterations for EP-Mediated Uptake

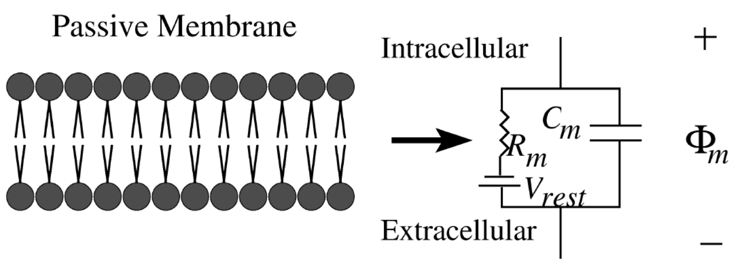
## Addition of current due to EP

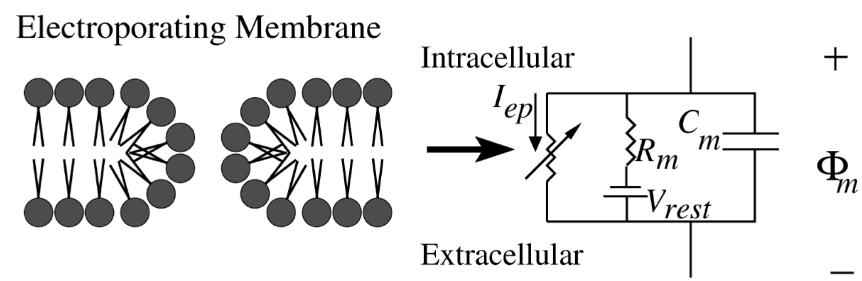



$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

# Alterations for EP-Mediated Uptake

## Addition of current due to EP



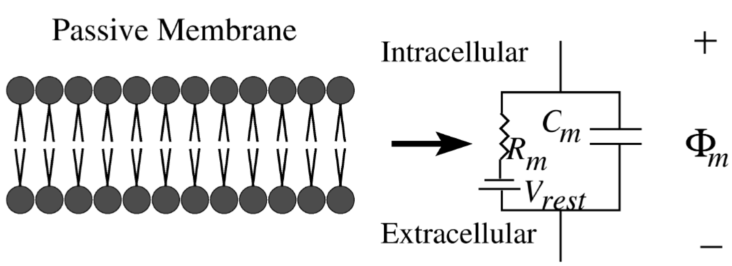



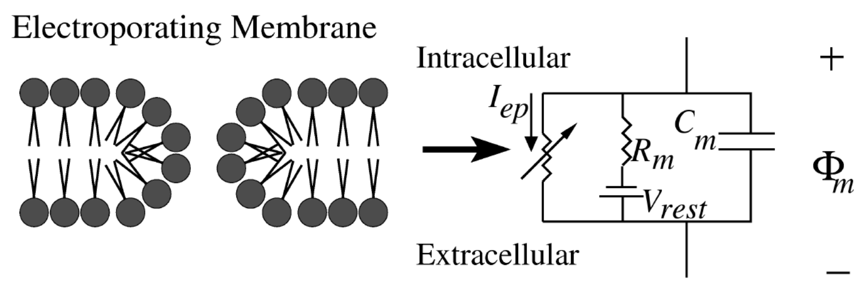

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m} + \boxed{I_{ep}}$$

# Alterations for EP-Mediated Uptake

## Addition of current due to EP



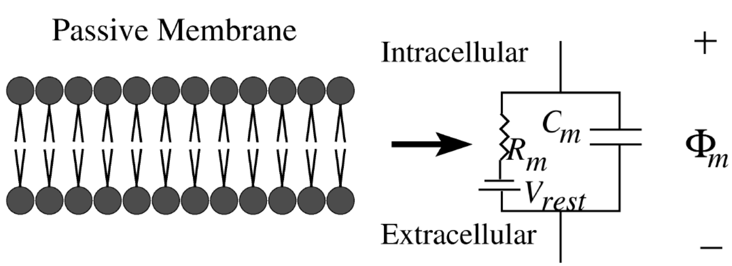

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

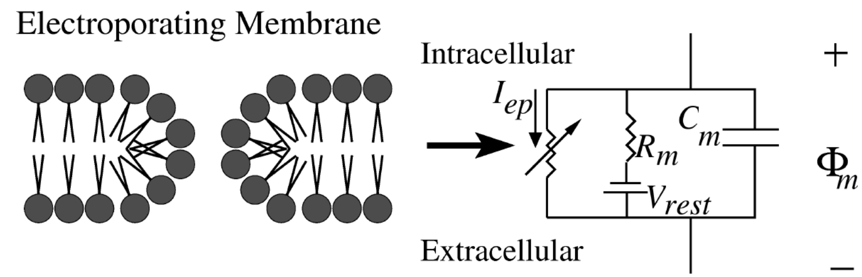
$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m} + I_{ep}$$

$$I_{ep} = N_{ep} i_{ep}$$

# Alterations for EP-Mediated Uptake

## Addition of current due to EP




$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m} + I_{ep}$$

$$I_{ep} = \boxed{N_{ep}} i_{ep}$$

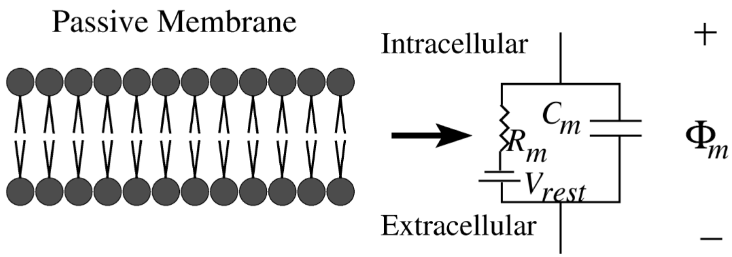
**Pore density**

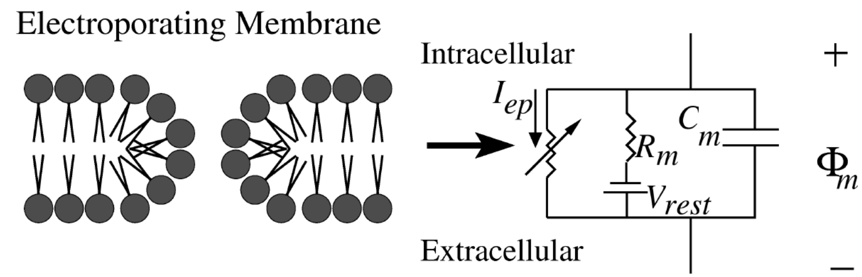
Published ODE model [1]
(highly nonlinear)

[1]Neu, J.C. & Krassowska, W. (1999) PRE, 59, 3471-3482

# Alterations for EP-Mediated Uptake

## Addition of current due to EP




$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m}$$

$$I_m(\Phi_m) = \frac{\Phi_m - V_{rest}}{R_m} + I_{ep}$$

$$I_{ep} = N_{ep}\boxed{i_{ep}}$$

### Pore density

Published ODE model [1]
(highly nonlinear)

### Current through an electropore

Published aqueous pore model[2]

[1]Neu, J.C. & Krassowska, W. (1999) PRE, 59, 3471-3482
[2]Barnett, A. (1999) Biochim. Biophys. Acta, 1025, 10-14

# Outline

## Introduction

- Background
- Published experiments and models

## **Derivation and Validation of Models**

- Asymptotic fiber model
- Alterations for EP-mediated uptake
- **Mass transport model**

## Results

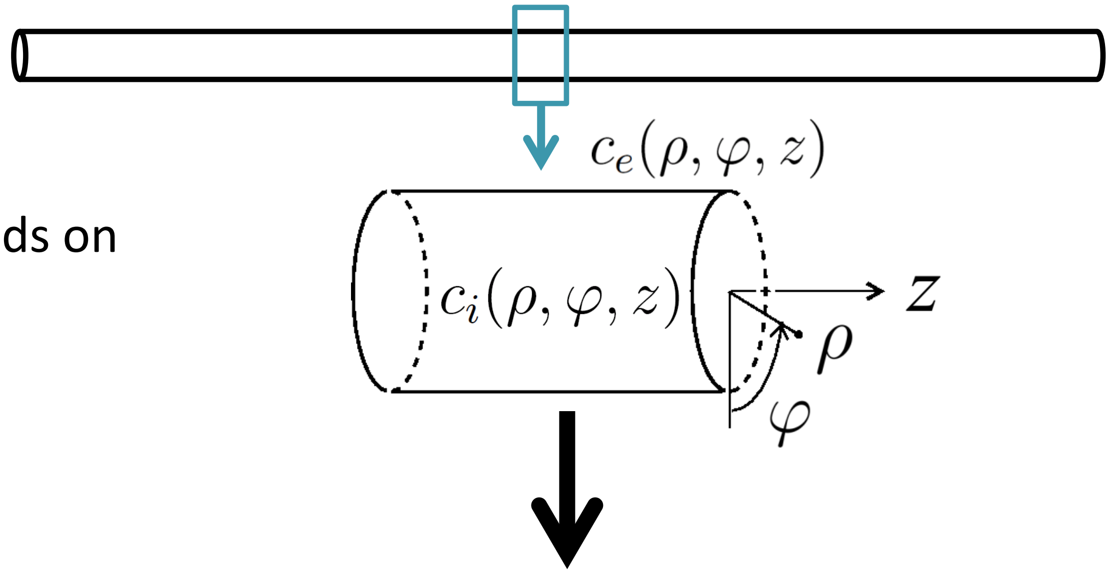
- Single fiber close to electrodes
- Single fiber far from electrodes
- Model variations and tissue-wide effect

## Conclusions and Connections to LLNL

# Derivation of Mass Transport Model

Model transport of molecules

Full 3D convection-diffusion equations



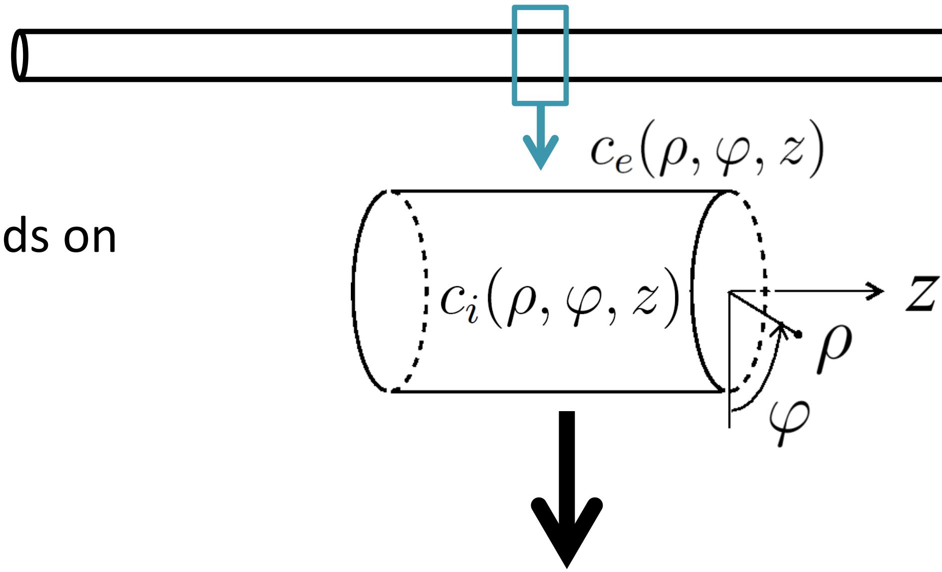

Transport depends on

- Electric drift
- Diffusion

# Derivation of Mass Transport Model

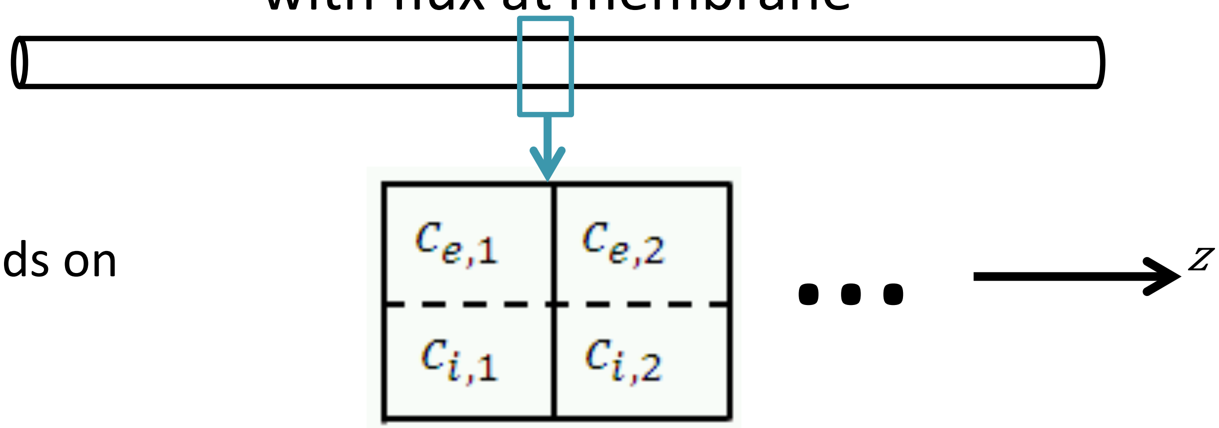
<u>Model transport of molecules</u>

Full 3D convection-diffusion equations




Transport depends on
- Electric drift
- Diffusion

Series of longitudinally independent, two-compartment equations with flux at membrane




Transport depends on
- Diffusion

# Beginning of Mass Transport Model Deriv.

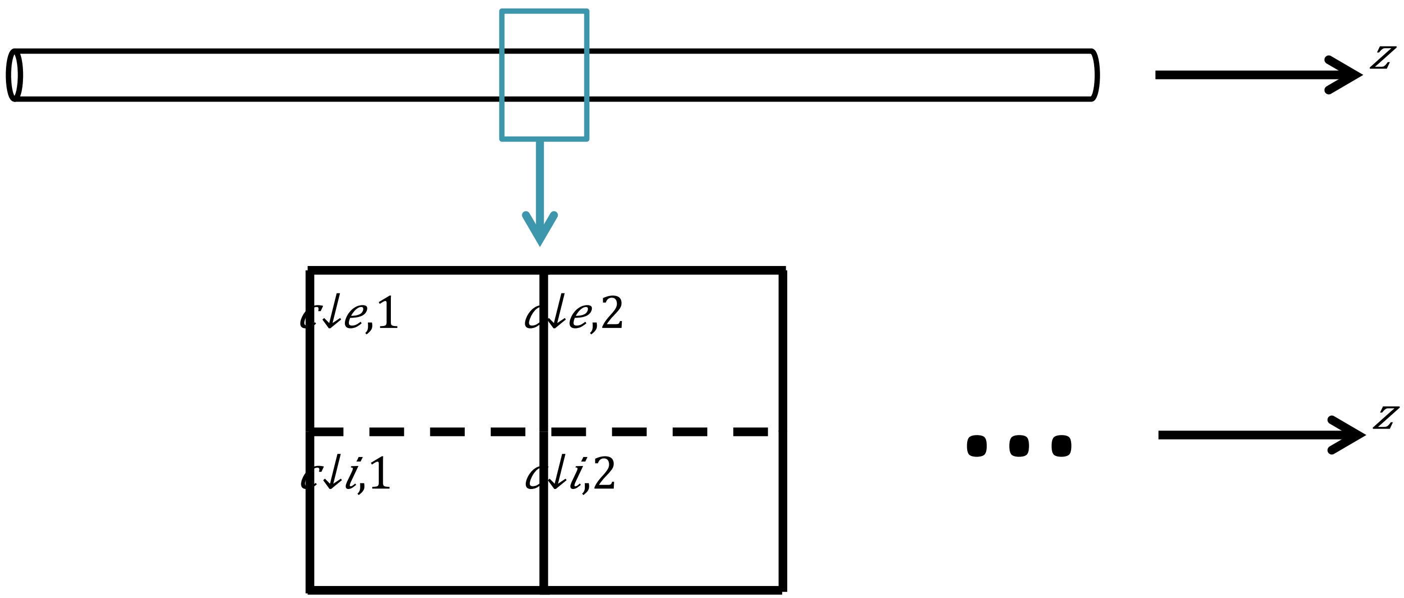
# Derivation of Mass Transport Model

Model transport of molecules

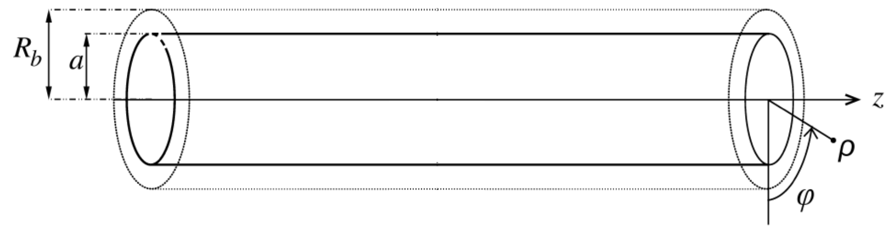
Full 3D convection-diffusion equations

↓

Series of longitudinally independent, two-compartment equations with flux at membrane



# Derivation of Mass Transport Model




## Convection-diffusion equation

$$\frac{\partial c_i}{\partial t} = \nabla \cdot \{D \nabla c_i - \mathbf{v_i} c_i\} \quad \text{in } \rho < a,$$

$$\frac{\partial c_e}{\partial t} = \nabla \cdot \{D \nabla c_e - \mathbf{v_e} c_e\} \quad \text{in } a < \rho < R_b$$

## Electric drift velocity

$$\mathbf{v_i} = -D \frac{z_m e}{k_B T} \nabla V_i \quad \text{in } \rho < a,$$

$$\mathbf{v_e} = -D \frac{z_m e}{k_B T} \nabla V_e \quad \text{in } a < \rho < R_b$$

## Continuity of molecular flux across membrane boundary

$$D \left\{ \frac{\partial c_i}{\partial \rho} + \frac{c_i z_m e}{k_B T} \frac{\partial V_i}{\partial \rho} \right\} = D \left\{ \frac{\partial c_e}{\partial \rho} + \frac{c_e z_m e}{k_B T} \frac{\partial V_e}{\partial \rho} \right\} = -j_m \quad \text{on } \rho = a$$

# Derivation of Mass Transport Model

## Molecule and pulsing parameters

| Parameter | Definition | Units | Value |
|---|---|---|---|
| $\|z_m\|$ | Valence magnitude | Unitless | 1 |
| $D$ | Diffusion coefficient | $m^2\ s^{-1}$ | $2.01 \times 10^{-10}$ |
| $T$ | Temperature | K | 310 |
| $\tau_p$ | Pulse duration | $\mu s$ | 100 |
| $\|\|E\|\|_\infty$ | Max. magnitude of field | $kV\ cm^{-1}$ | 10 |
| $N$ | Number of pulses | Unitless | 8 |
| $T_p$ | Period of pulse train | s | 1 |
| $a$ | Fiber radius | $\mu m$ | 25 |

# Derivation of Mass Transport Model

## Molecule and pulsing parameters

| Parameter | Definition | Units | Value |
|---|---|---|---|
| $\|z_m\|$ | Valence magnitude | Unitless | 1 |
| $D$ | Diffusion coefficient | m$^2$ s$^{-1}$ | 2.01 x 10$^{-10}$ |
| $T$ | Temperature | K | 310 |
| $\tau_p$ | Pulse duration | $\mu$s | 100 |
| $\|\|E\|\|_\infty$ | Max. magnitude of field | kV cm$^{-1}$ | 10 |
| $N$ | Number of pulses | Unitless | 8 |
| $T_p$ | Period of pulse train | s | 1 |
| $a$ | Fiber radius | $\mu$m | 25 |

## Two time scales

1. Electric drift: $N\tau_p = 800\ \mu\text{s}$
2. Diffusion: 8.5 s

# Derivation of Mass Transport Model

## Molecule and pulsing parameters

| Parameter | Definition | Units | Value |
|---|---|---|---|
| $\|z_m\|$ | Valence magnitude | Unitless | 1 |
| $D$ | Diffusion coefficient | $m^2\ s^{-1}$ | $2.01 \times 10^{-10}$ |
| $T$ | Temperature | K | 310 |
| $\tau_p$ | Pulse duration | $\mu s$ | 100 |
| $\|\|E\|\|_\infty$ | Max. magnitude of field | $kV\ cm^{-1}$ | 10 |
| $N$ | Number of pulses | Unitless | 8 |
| $T_p$ | Period of pulse train | s | 1 |
| $a$ | Fiber radius | $\mu m$ | 25 |

## Two time scales

1. Electric drift: $N\tau_p = 800\ \mu s$
2. Diffusion: 8.5 s

## Two length scales

1. Electric drift: $L_e = \underbrace{\left(\frac{D|z_m|e}{k_B T}||E||_\infty\right)}_{\text{max. velocity}} 800\mu s = 6.01\ \mu m$
2. Diffusion: $L_d = \sqrt{6D8.5\ s} = 101\ \mu m$

# Derivation of Mass Transport Model

## Molecule and pulsing parameters

| Parameter | Definition | Units | Value |
|---|---|---|---|
| $\|z_m\|$ | Valence magnitude | Unitless | 1 |
| $D$ | Diffusion coefficient | $m^2\ s^{-1}$ | $2.01 \times 10^{-10}$ |
| $T$ | Temperature | K | 310 |
| $\tau_p$ | Pulse duration | $\mu s$ | 100 |
| $\|\|E\|\|_\infty$ | Max. magnitude of field | $kV\ cm^{-1}$ | 10 |
| $N$ | Number of pulses | Unitless | 8 |
| $T_p$ | Period of pulse train | s | 1 |
| $a$ | Fiber radius | $\mu m$ | 25 |

## Two time scales

1. Electric drift: $N\tau_p = 800\ \mu s$
2. Diffusion: 8.5 s

## Two length scales

1. Electric drift: $L_e = \underbrace{\left(\frac{D|z_m|e}{k_B T}||E||_\infty\right)}_{\text{max. velocity}} 800\mu s = 6.01\ \mu m$

2. Diffusion: $L_d = \sqrt{6D8.5\ s} = 101\ \mu m$

## Two assumptions

1. Electric drift ignored ($L_e$ is 6% of $L_d$)
2. "Well-mixed" in transverse direction ($L_d$ is four-fold larger than fiber radius $a$ )

# Derivation of Mass Transport Model

Reduction to two-compartment, 1D longitudinal diffusion equations

Divergence theorem

- Neglect electric drift
- Uniform transverse concentration

$$\frac{\partial c_i}{\partial t} = D\frac{\partial^2 c_i}{\partial z^2} - \frac{2}{a}\overline{j_m},$$

$$\frac{\partial c_e}{\partial t} = D\frac{\partial^2 c_e}{\partial z^2} + \frac{2a}{R_b^2 - a^2}\overline{j_m}$$

$j_m$ is instantaneous function of $c_i$, $c_e$, and $N_{ep}$

# Derivation of Mass Transport Model

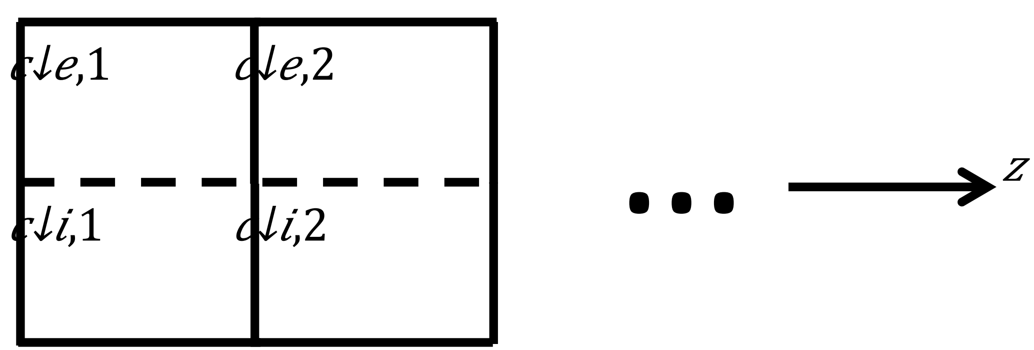
Remove longitudinal diffusion

$$\frac{\partial c_i}{\partial t} = \cancel{D\frac{\partial^2 c_i}{\partial z^2}} - \frac{2}{a}\overline{j_m},$$

$$\frac{\partial c_e}{\partial t} = \cancel{D\frac{\partial^2 c_e}{\partial z^2}} + \frac{2a}{R_b^2 - a^2}\overline{j_m}$$

Maximum error in total uptake in entire fiber from neglecting longitudinal diffusion is 0.1%

$$\boxed{\begin{aligned} \frac{\partial c_i}{\partial t} &= -\frac{2}{a}\overline{j_m}, \\ \frac{\partial c_e}{\partial t} &= \frac{2a}{R_b^2 - a^2}\overline{j_m} \end{aligned}}$$



# End of Mass Transport Model Deriv.

# Outline

## Introduction

- Background
- Published experiments and models

## Derivation and Validation of Models

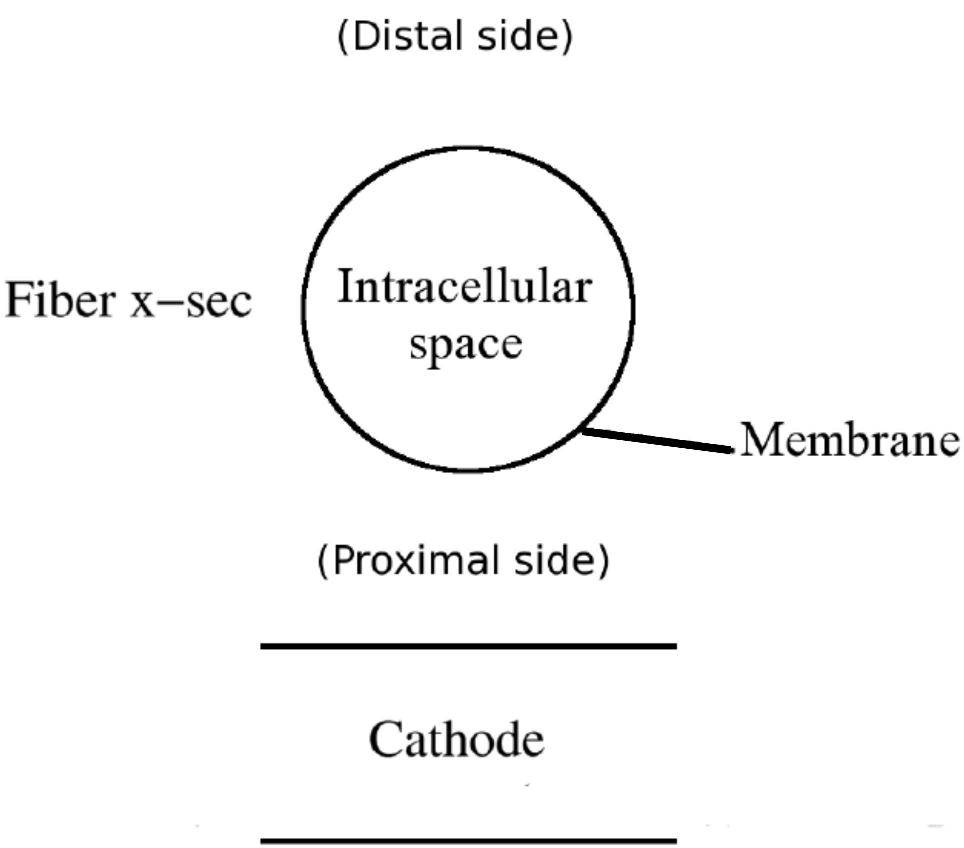
- Asymptotic fiber model
- Alterations for EP-mediated uptake
- Mass transport model

## **Results**

- **Single fiber close to electrodes**
- Single fiber far from electrodes
- Model variations and tissue-wide effect

## Conclusions and Connections to LLNL

# Passive Versus Electroporating




**Contour plots viewed at 3 time instants**

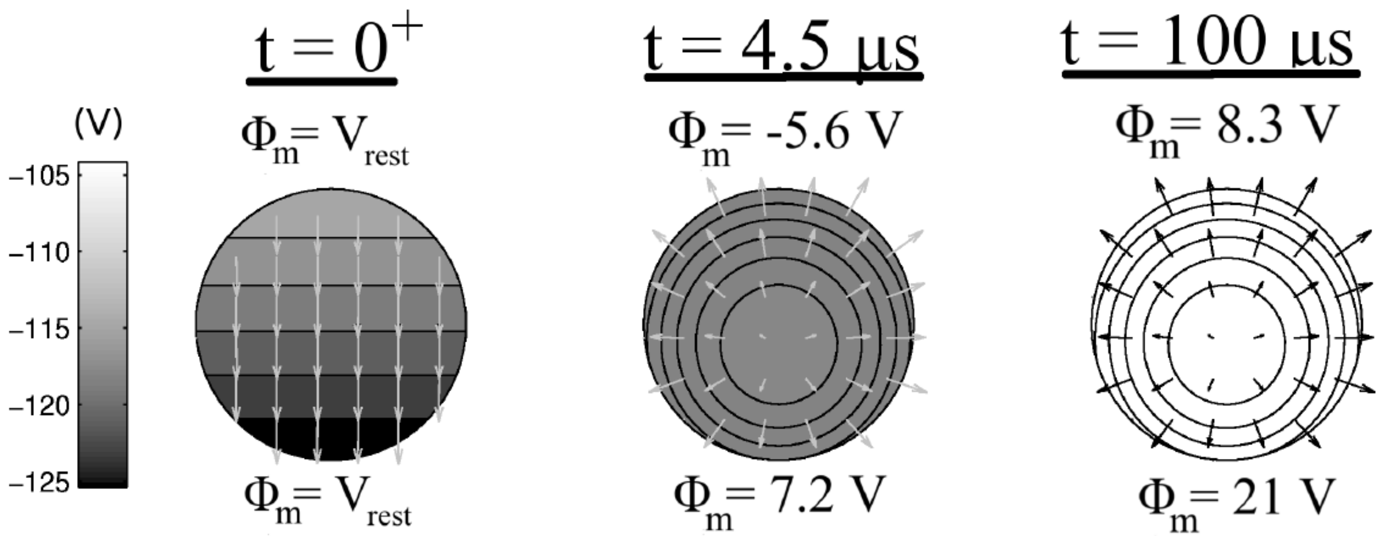
1. $t=0\uparrow+$ , when pulse turned on

2. $t=4.5\ \mu$s, ten-fold larger than time scale of membrane charging via transverse currents

3. $t=100\ \mu$s, pulse duration

# Passive Versus Electroporating

## Distribution of potential and current density

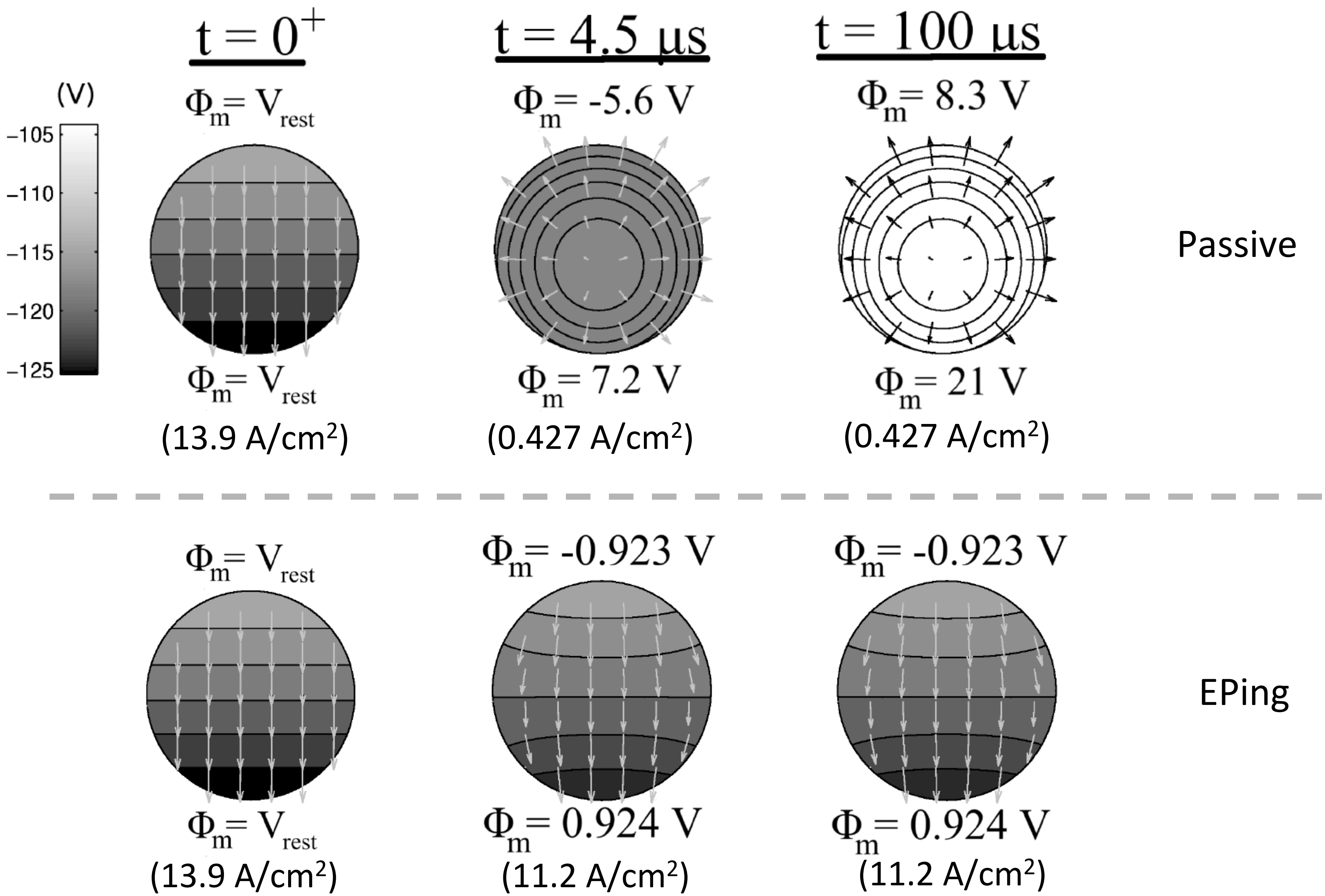



($13.9\ A/cm^2$) ($0.427\ A/cm^2$) ($0.427\ A/cm^2$)

Passive

# Passive Versus Electroporating

## Distribution of potential and current density



# Passive Versus Electroporating

<u>1D longitudinal problem for mean potential</u>

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t} = \left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2 \langle\Psi\rangle}{\partial z^2} - \left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

# Passive Versus Electroporating

## 1D longitudinal problem for mean potential

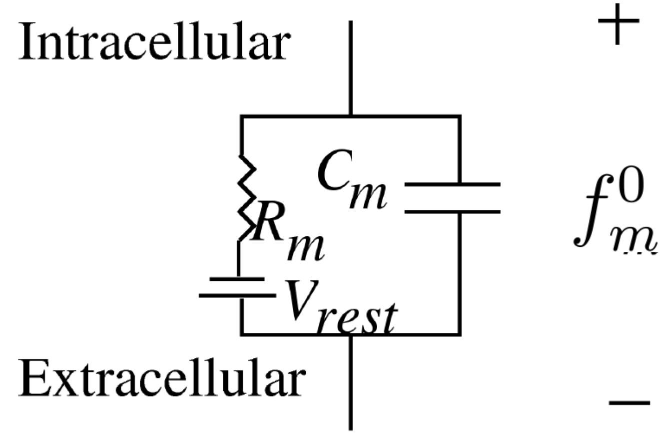
$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t} = \left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\langle\Psi\rangle}{\partial z^2} - \left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

Passive fiber

$$\overline{I_m} = \left(\frac{1}{R_m}\right)f_m^0 - \frac{V_{rest}}{R_m}$$

$$\downarrow$$

**Time scale charging: 10 milliseconds**



# Passive Versus Electroporating

## 1D longitudinal problem for mean potential

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t} = \left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\langle\Psi\rangle}{\partial z^2} - \left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

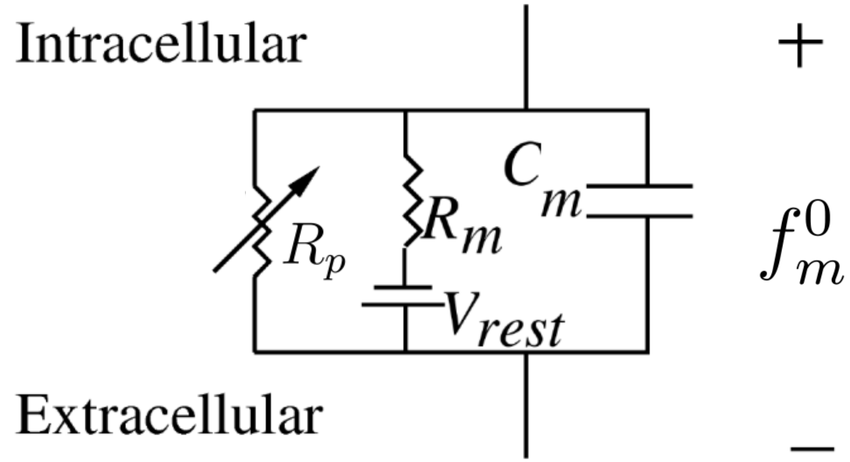
Electroporating fiber

$$\overline{I_m} = \left(\frac{R_m+R_p}{R_m R_p}\right)f_m^0 - \frac{V_{rest}}{R_m}$$

- Choose typical value of $R_p$ that develops on short time scale due to transverse charging

↓

**Time scale of charging: 1 microsecond**



# Passive Versus Electroporating

## 1D longitudinal problem for mean potential

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t} = \left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\langle\Psi\rangle}{\partial z^2} - \left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

Passive fiber

**10 milliseconds**

Membrane continues charging after 4.5 $\mu$s

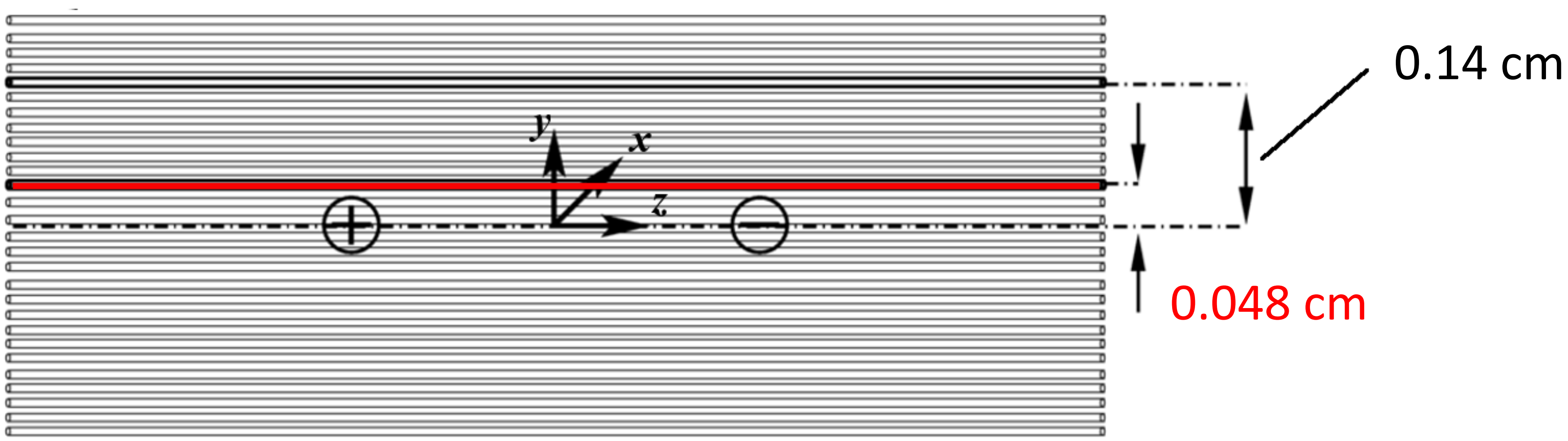
Electroporating fiber

**1 microseconds**

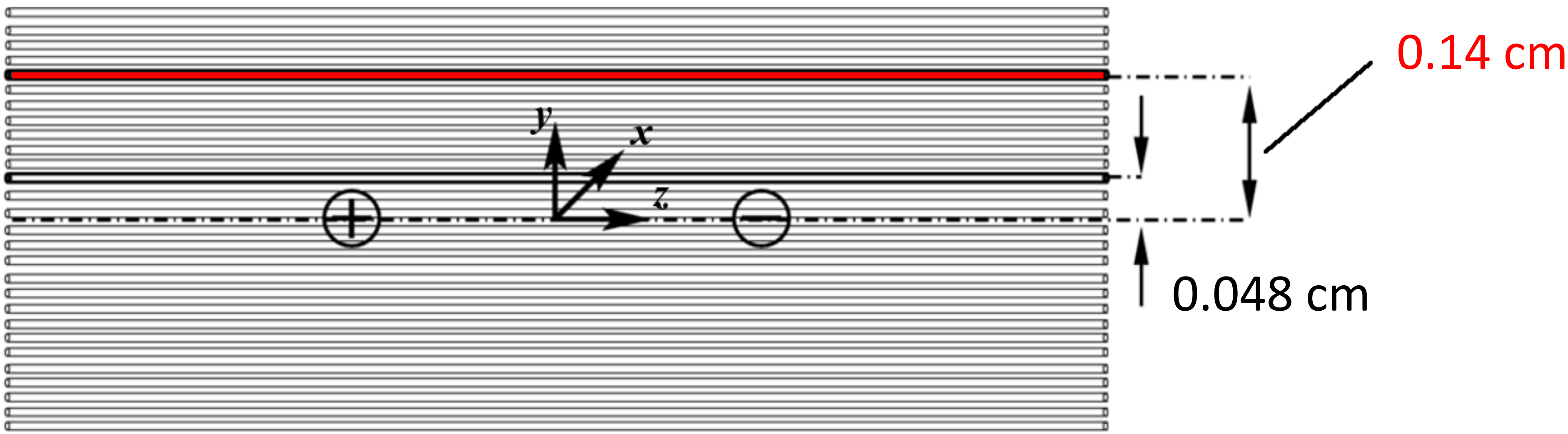
Membrane stops charging and EPing after 4.5 $\mu$s

**If EP occurs on time scale transverse charging (4.5 ,**
**it precludes further charging and pore creation in longitudinal problem**

# Fiber Close to Electrodes

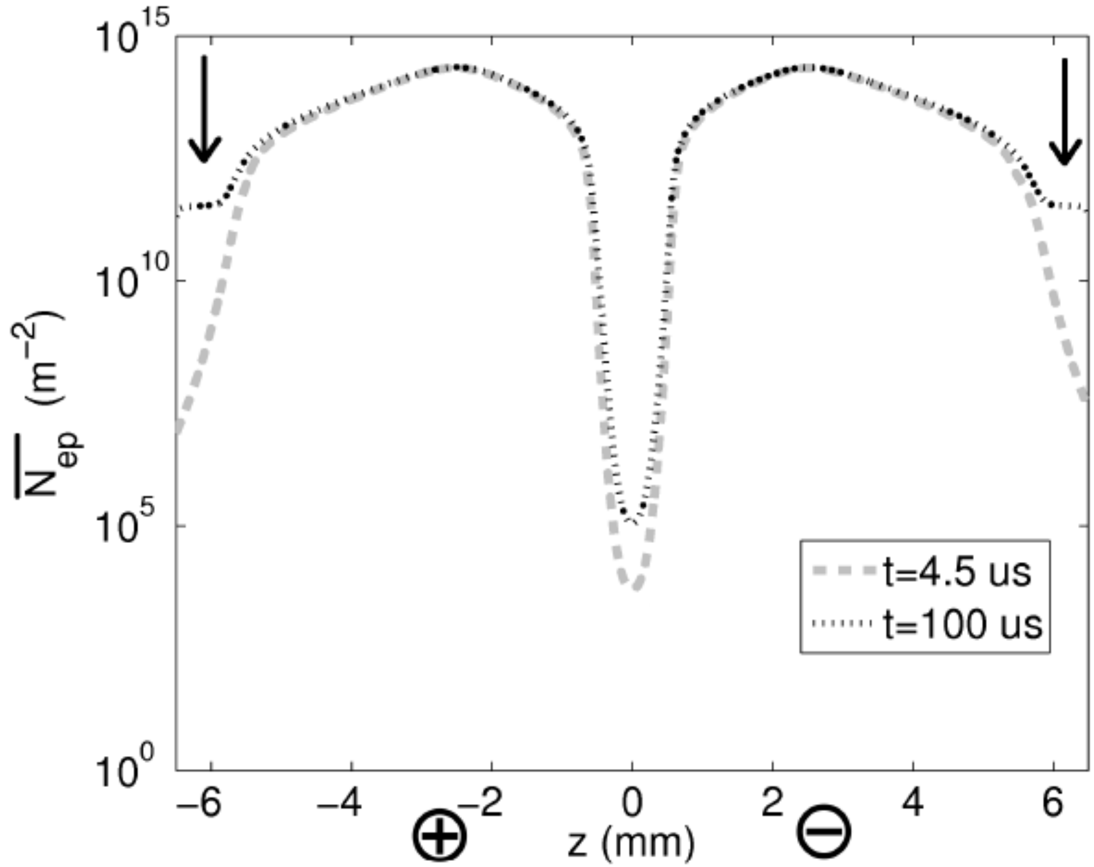


# Fiber Far From Electrodes



# Electroporating Fiber

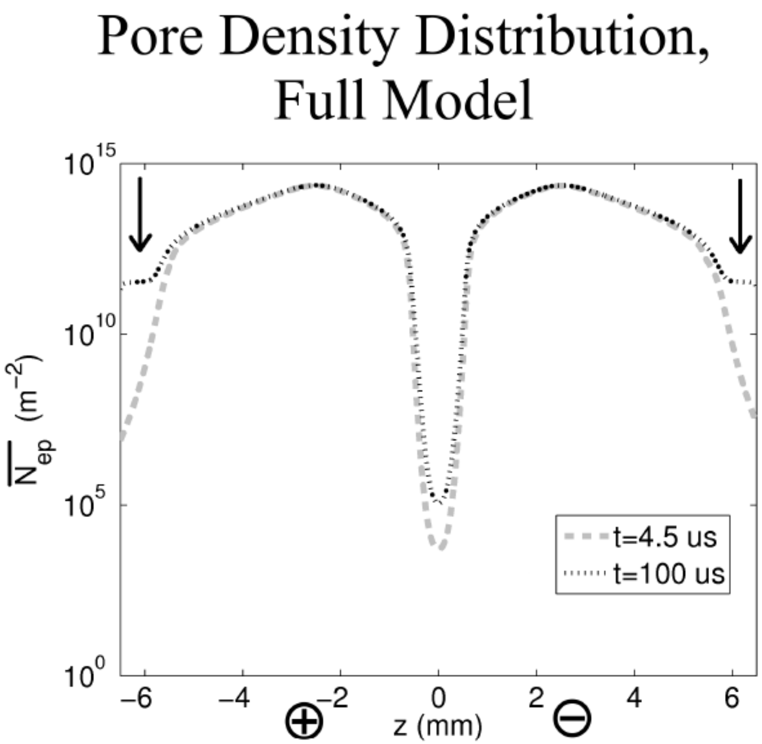
<u>Does charging and EPing cease after 4.5$\mu$s over the entire fiber?</u>



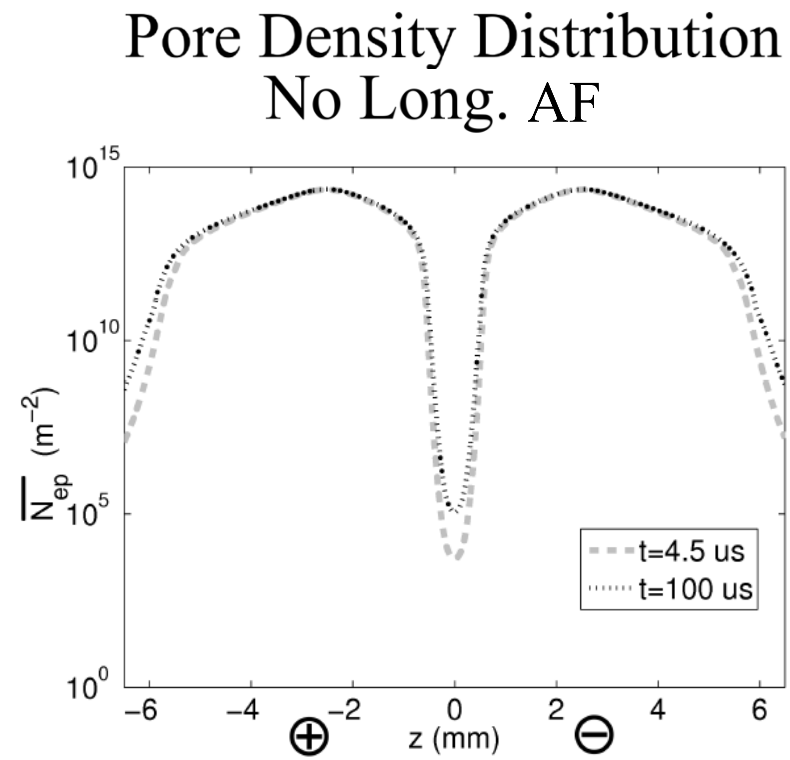

Membrane charging and additional pore creation after 4.5 $\mu$s for $|z|>6$ mm

# Effect of Longitudinal Charging

<u>Creation of pores past 4.5 $\mu$s from transverse or longitudinal charging?</u>



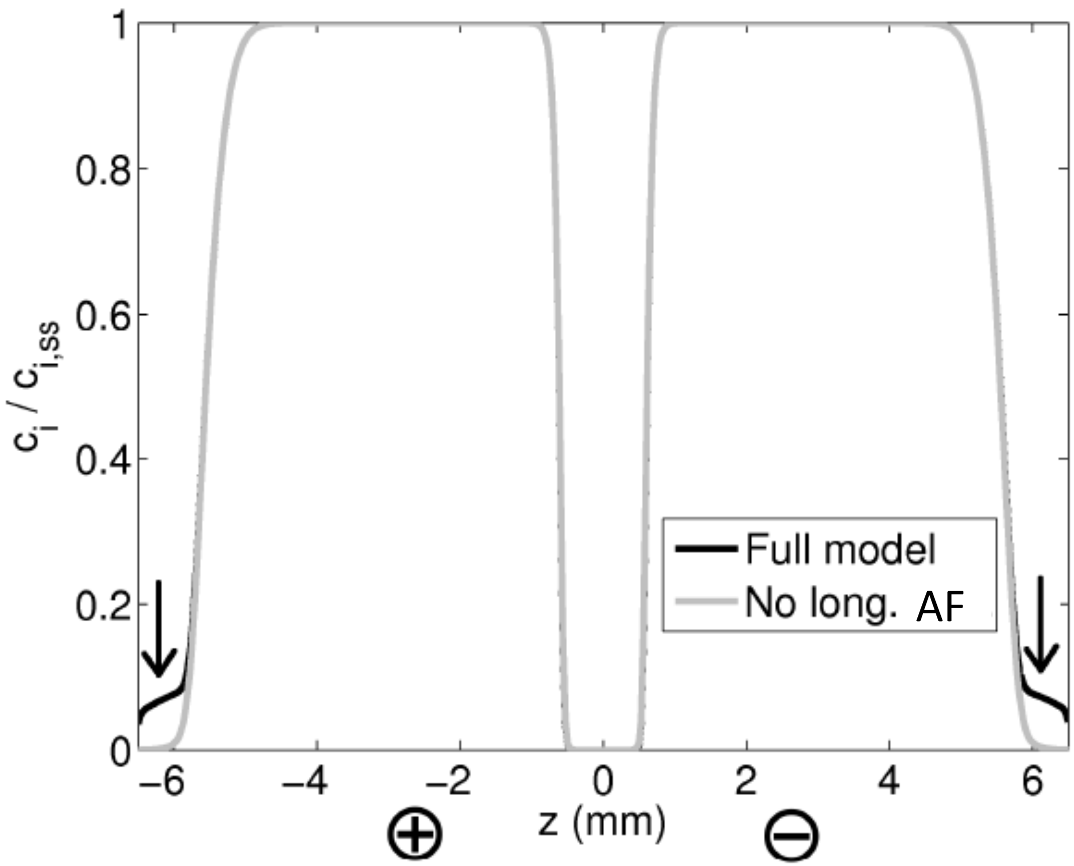

Charging and addition pore creation after 4.5 $\mu$s for $|z|>6$ mm




No charging and addition pore creation after 4.5 $\mu$s for $|z|>6$ mm

# Effect of Longitudinal Charging

## Effect of longitudinal charging on uptake




Longitudinal AF effects uptake only for $|z|>6$ mm

**Difference in total uptake over entire fiber is 0.91 %**

# Outline

## Introduction

- Background
- Published experiments and models

## Derivation and Validation of Models

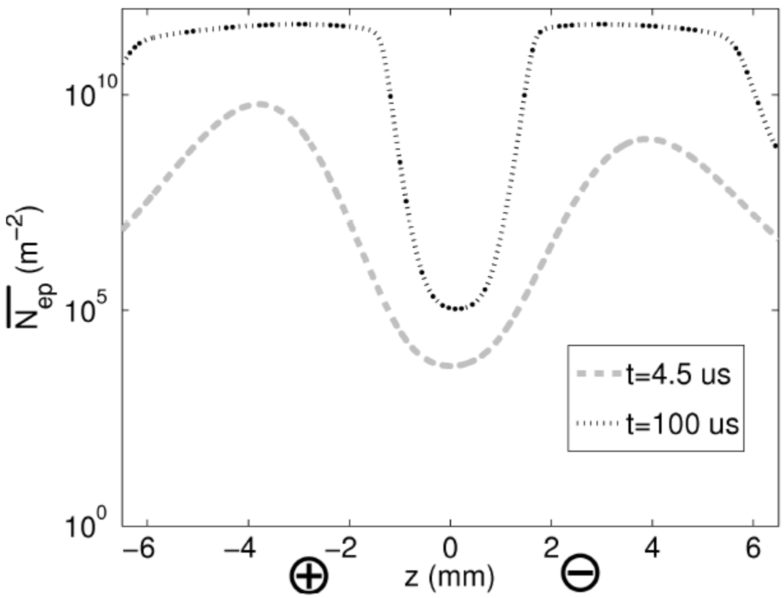
- Asymptotic fiber model
- Alterations for EP-mediated uptake
- Mass transport model

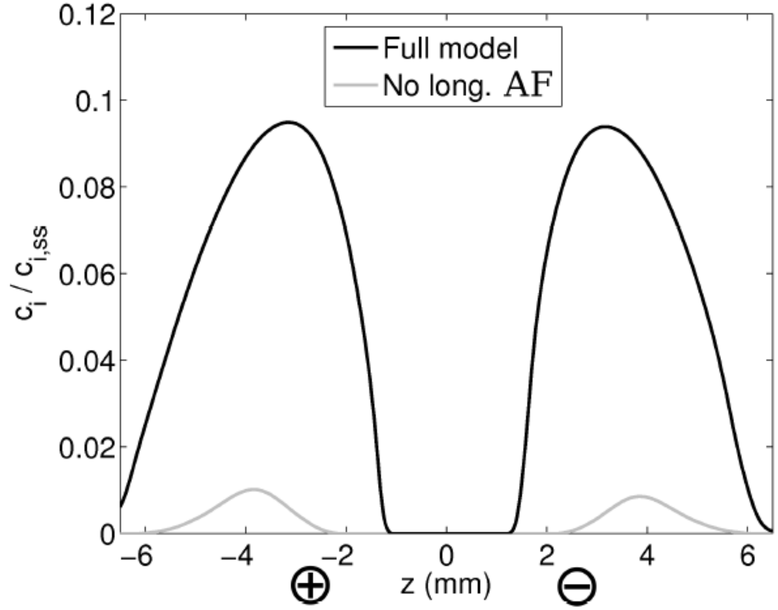
## **Results**

- Single fiber close to electrodes
- **Single fiber far from electrodes**
- Model variations and tissue-wide effect

## Conclusions and Connections to LLNL

# Effect of Longitudinal Charging




Membrane charging and additional pore creation after 4.5 $\mu$s for all locations

Longitudinal AF effects uptake for all positions

**Difference in total uptake over entire fiber is 2000%**

# Passive Versus Electroporating

1D longitudinal problem for mean potential

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t}=\cancel{\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}}+\left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\left\langle\Psi\right\rangle}{\partial z^2}-\left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

# Effect of Longitudinal Charging

<u>Creation of pores past 4.5 $\mu$s from transverse or longitudinal charging?</u>

Simulate:

- Full transverse problem
- Full longitudinal problem

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t}=\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}+\left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\langle\Psi\rangle}{\partial z^2}-\left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

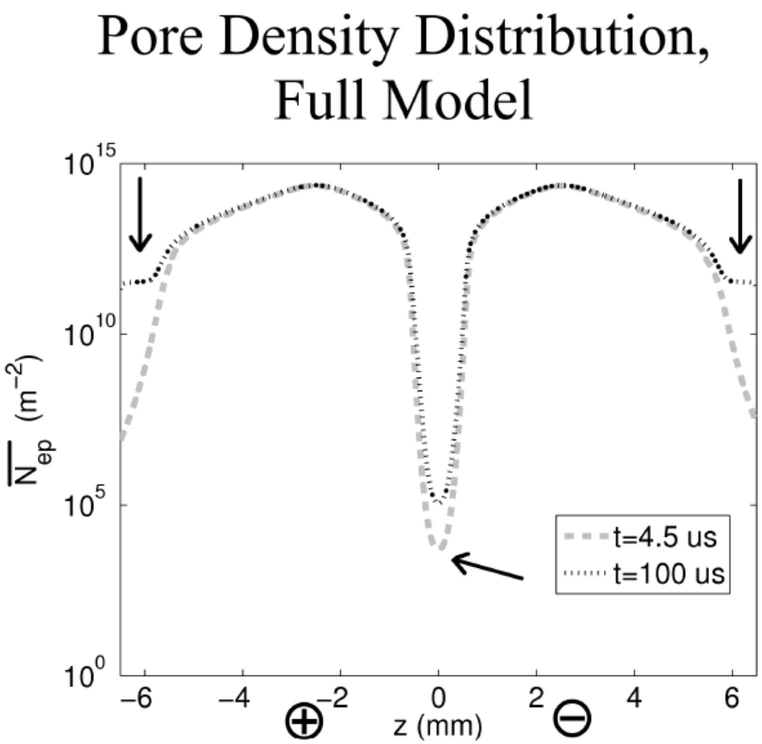
Simulate:

- Full transverse problem
- Partial longitudinal problem – no longitudinal AF

$$C_m\frac{\partial f_m^0}{\partial t}=-\overline{I_m}$$

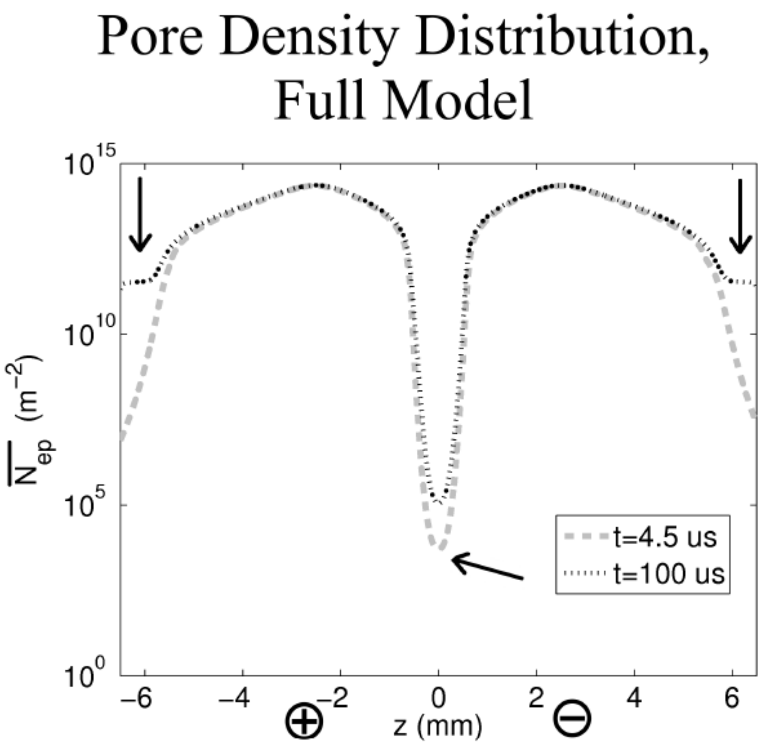
# Effect of Longitudinal Charging

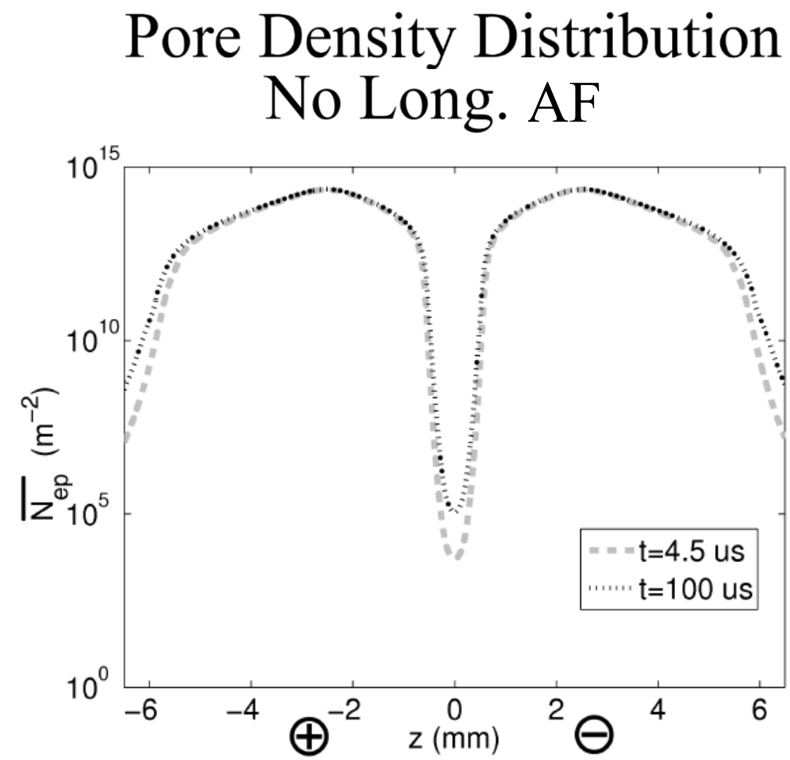
<u>Creation of pores past 4.5 $\mu$s from transverse or longitudinal charging?</u>



# Effect of Longitudinal Charging

<u>Creation of pores past 4.5 $\mu$s from transverse or longitudinal charging?</u>

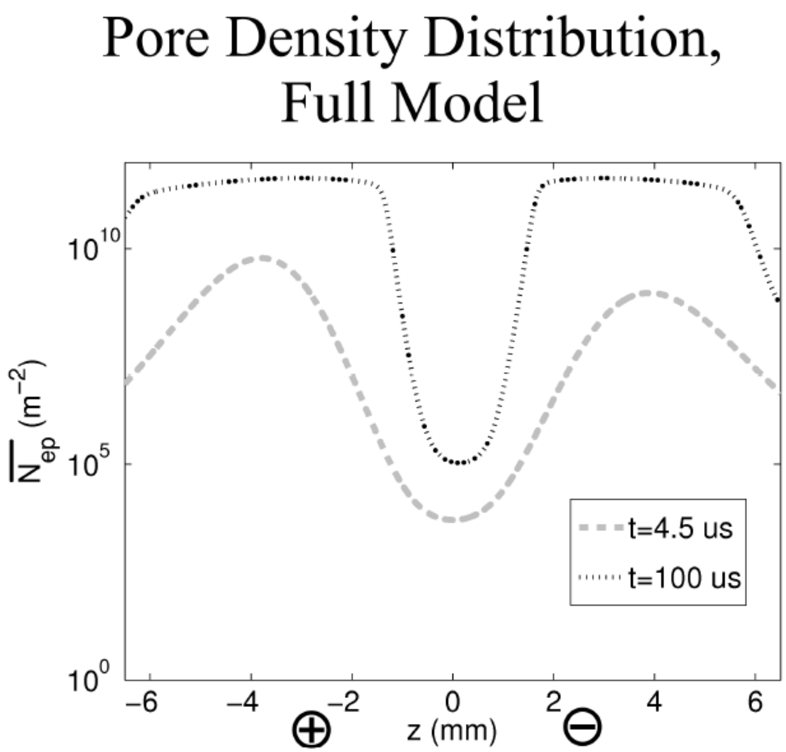



Charging and addition pore creation after 4.5 $\mu$s for $|z|>6$ mm

No charging and addition pore creation after 4.5 $\mu$s for $|z|>6$ mm

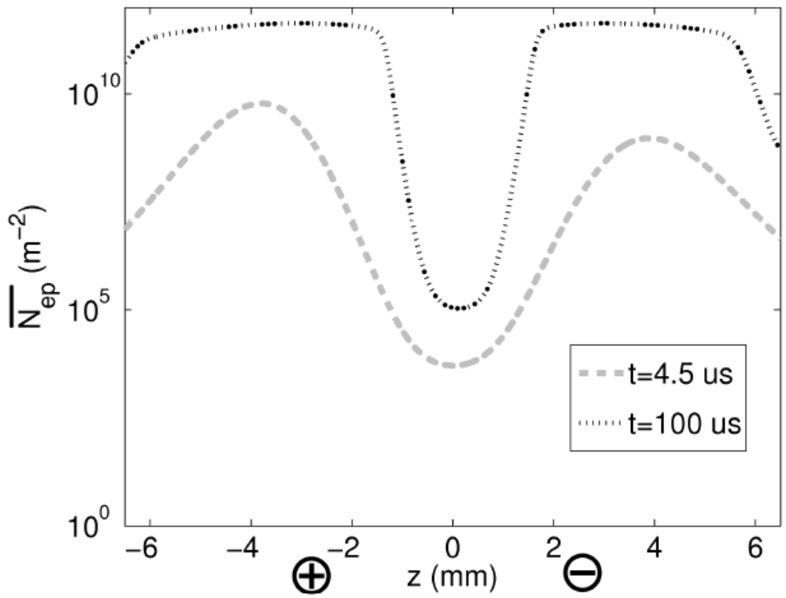
# Effect of Longitudinal Charging




Membrane charging and additional pore creation after 4.5 $\mu$s for all locations

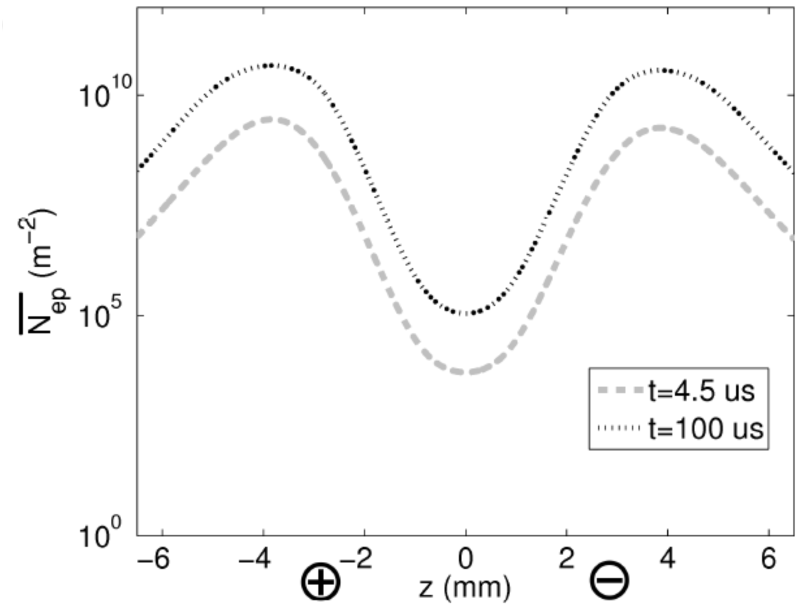
# Effect of Longitudinal Charging

Pore Density Distribution,
Full Model




Membrane charging and additional pore creation after 4.5 $\mu$s for all locations

Pore Density Distribution
No Long. AF




Membrane charging and additional pore creation after 4.5 $\mu$s for all locations, but $\overline{N_{ep}}$ at peaks is one order mag. smaller

# Outline

## Introduction

- Background
- Published experiments and models

## Derivation and Validation of Models

- Asymptotic fiber model
- Alterations for EP-mediated uptake
- Mass transport model

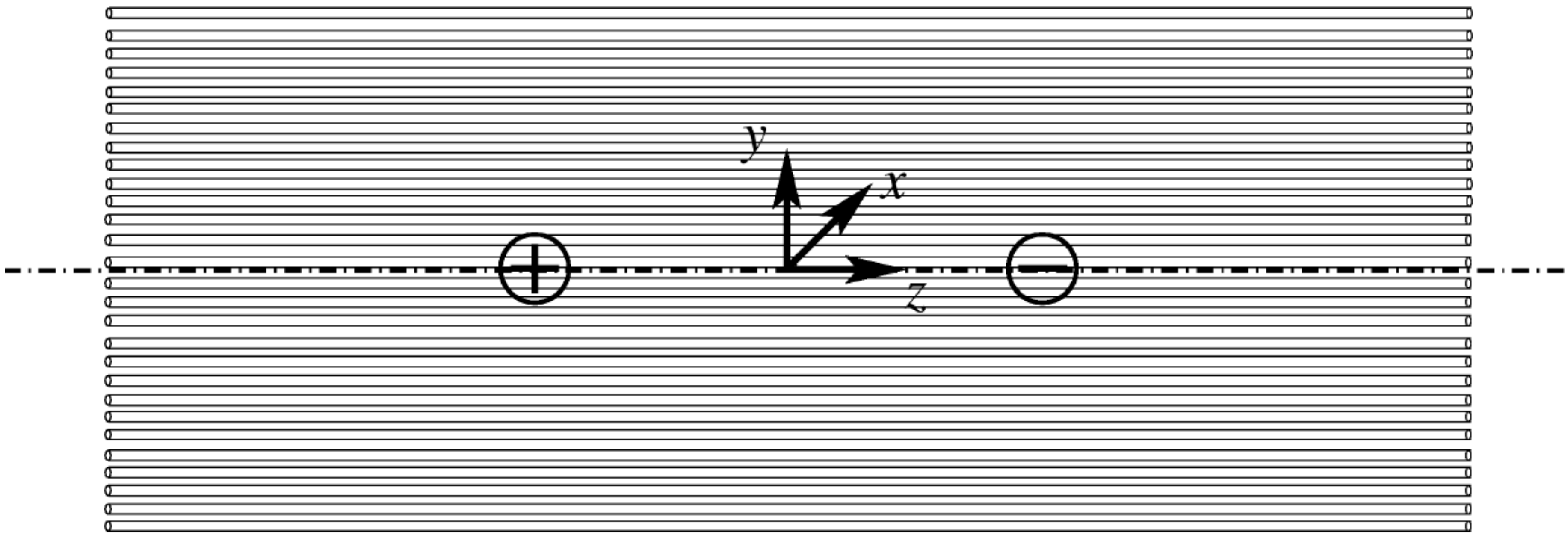
## **Results**

- Single fiber close to electrodes
- Single fiber far from electrodes
- **Model variations and tissue-wide effect**

## Conclusions and Connections to LLNL

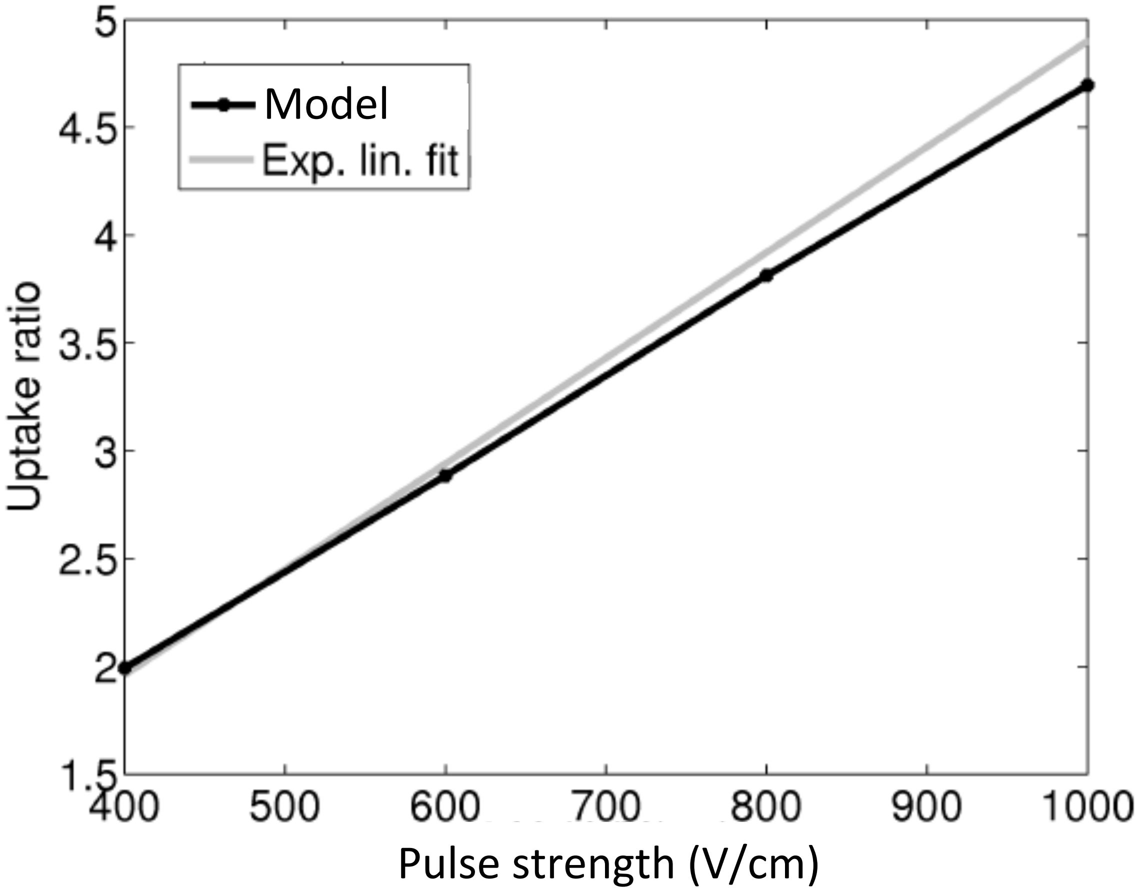
# Model Variations

Uptake over entire tissue



# Model Variations

<u>Pulse strength: comparison to experiments[1]</u>




Relative difference in uptake never exceeds 5%

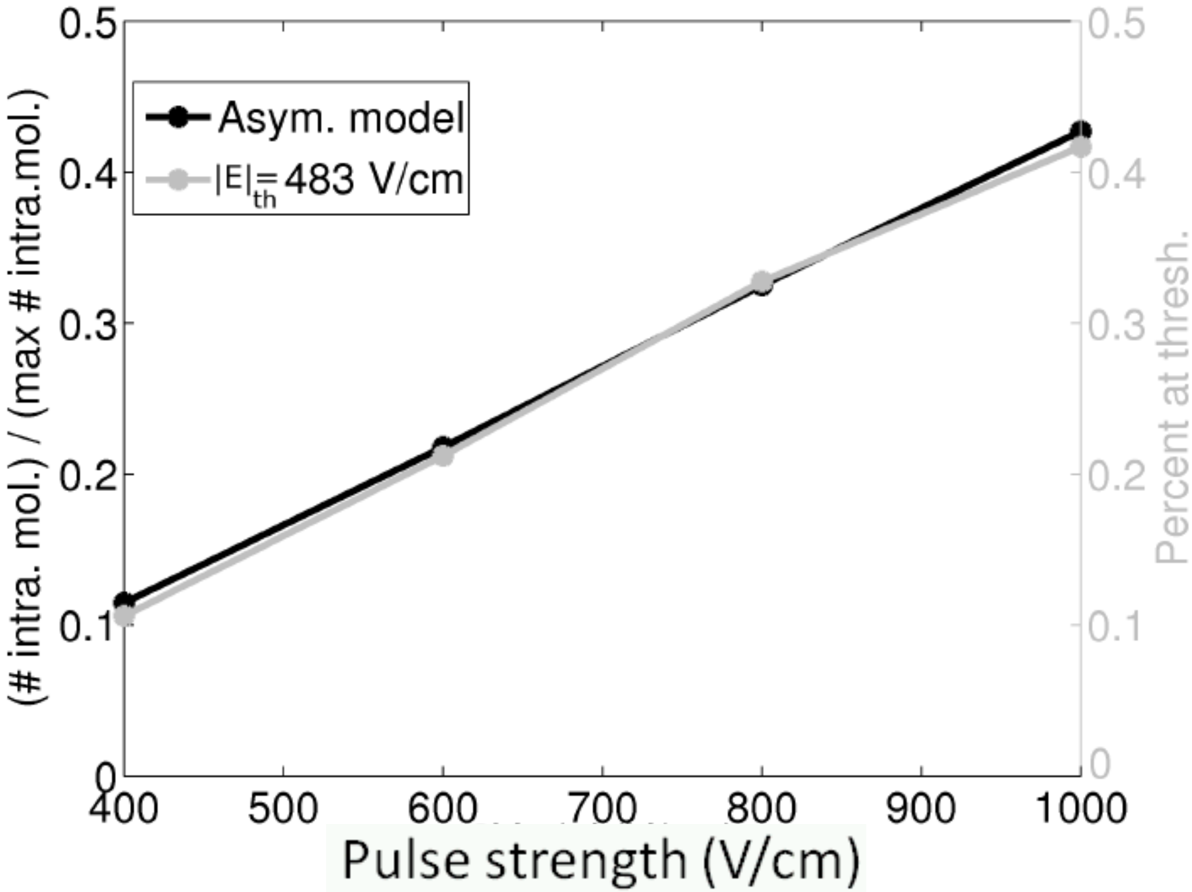
Experimental variability: 6%

Maximum contribution from longitudinal AF in this range of pulse strengths: 10%

*[1]Grafstrom,G., Engstrom,P., Salford,L.G.,Persson,B.R.R. (2006) Cancer Biother. Radio., 21,*

# Model Variations

## Pulse strength: comparison to threshold field model

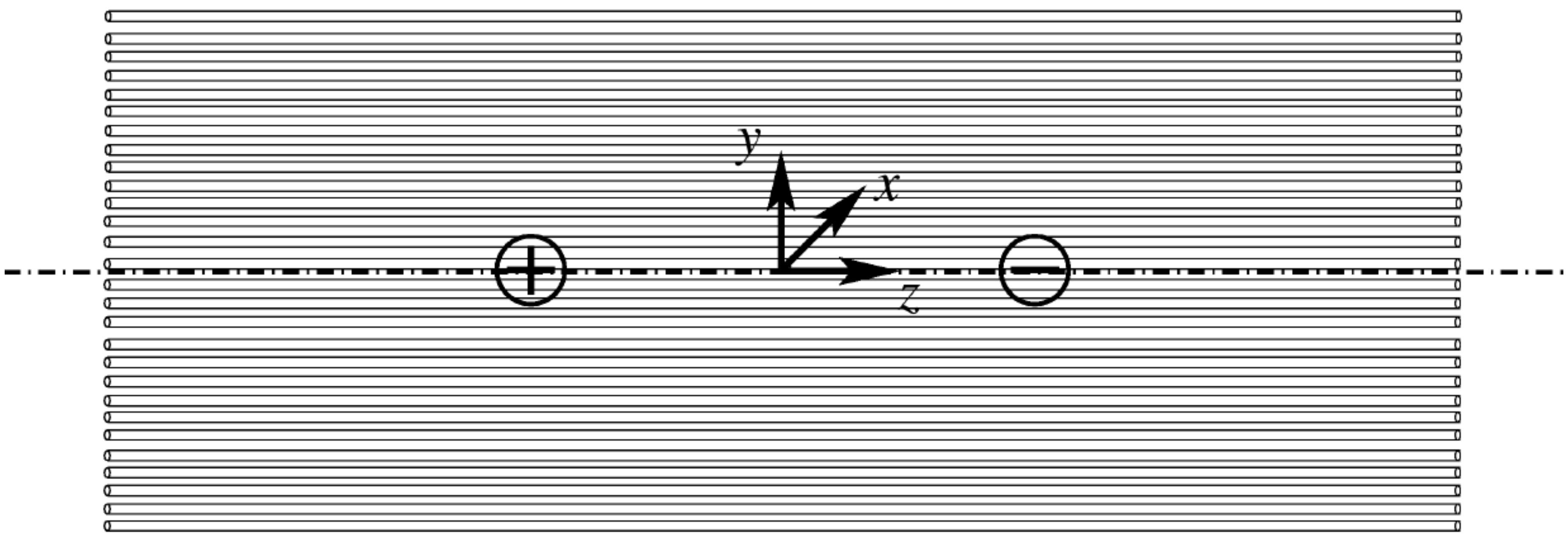



Average relative difference is 3.31%

Maximum relative difference is 7.45% at pulse strength 400 V/cm

# Model Variations

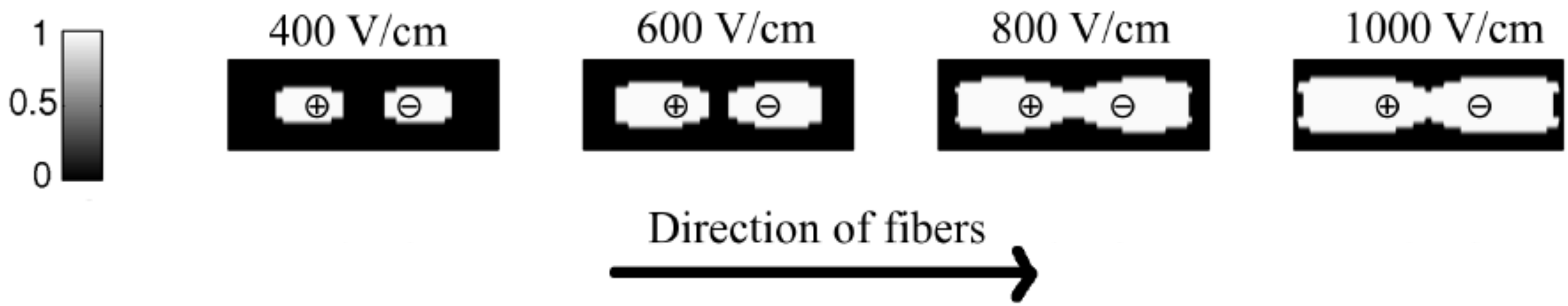
Muscle Tissue View



# Model Variations

## Comparison to threshold field model

### Threshold field model




### Asymptotic fiber model


Difference in uptake between electrodes

Relative root-mean-square difference around 20%

Correlation coefficient at most 0.969

# Outline

## Introduction

- Background
- Published experiments and models

## Derivation and Validation of Models

- Asymptotic fiber model
- Alterations for EP-mediated uptake
- Mass transport model

## Results

- Single fiber close to electrodes
- Single fiber far from electrodes
- Model variations and tissue-wide effect

## **Conclusions and Connections to LLNL**

# Conclusions

## Fiber close to electrodes

1. Significant EP occurs on the time scale of transverse charging
2. Charging of the membrane and creation of pores from longitudinal AF is impeded

## Fiber far from electrodes

1. Minimal EP occurs on time scale of transverse charging
2. Majority of membrane charging and creation of pores attributed to longitudinal AF

## Uptake over entire tissue

- Contribution of longitudinal AF never more than 10%
- 10% on order of experimental variability
- Corroborates previous models in terms of total uptake, but not distribution

## Reproduce tissue uptake experiments qualitatively and quantitatively

# Model Variations

<u>Pulse strength: comparison to threshold field model</u>

- Other models base electroporation off of field magnitude $|E|$

- Assume electroporating when $|E| > |E|_{th}$

- Formulate tissue conductivity, and corresponding uptake, based on choice of $|E|_{th}$

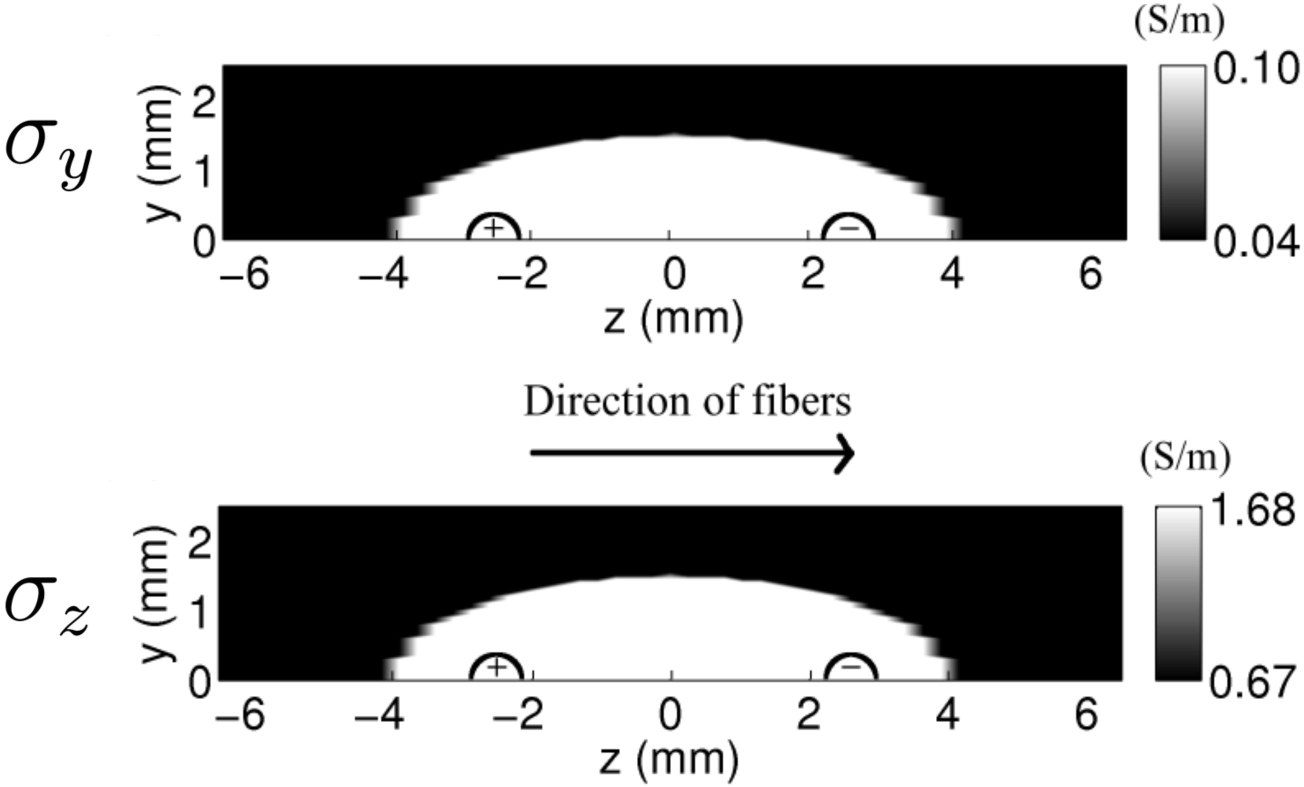
# Model Variations

## Effect of electroporation on tissue conductivities

Old way: Uniform macroscopic tissue conductivities

New way: Nonuniform macroscopic tissue conductivities

$$\sigma_y \frac{\partial^2 \Psi}{\partial y^2} + \sigma_z \frac{\partial^2 \Psi}{\partial z^2} = 0$$




Region of elevated conductivities approximates experimentally-validated regions

Conductivities increase by 2.5[b]

[a] *Sel, D., Cukjati, D. Batiuskaite, D., Slivnik, T., Mir, L. M. Miklavcic, D. (2005) IEEE T. Bio-Med. Eng., 52, 816-827*

[b] *Corovic, S., Lackovic, I., Sustaric, P., Sustar, T., Rodic, T., Miklavcic, D. (2013) Biomed. Eng. Online, 12:16*

# Model Variations

Effect of electroporation on tissue conductivities

Simulate two versions solving $\Psi$ numerically

1. Uniform tissue conductivities
2. Nonuniform tissue conductivities

Find numerical transverse AF $\partial\tilde{\Psi}/\partial\rho$ and longitudinal AF $\partial^2 \left\langle\Psi\right\rangle /\partial z^2$

Nonuniform version

3.51% increase in uptake from longitudinal AF

Uniform version

3.77% increase in uptake from longitudinal AF

Neglecting effect of EP on tissue conductivities does not change qualitative role of longitudinal AF

# Model Variations

<u>Effect of electroporation on tissue conductivities</u>

Total uptake: nonuniform version predicts 8.3% more uptake than uniform version

# Model Variations

## Effect of electroporation on tissue conductivities

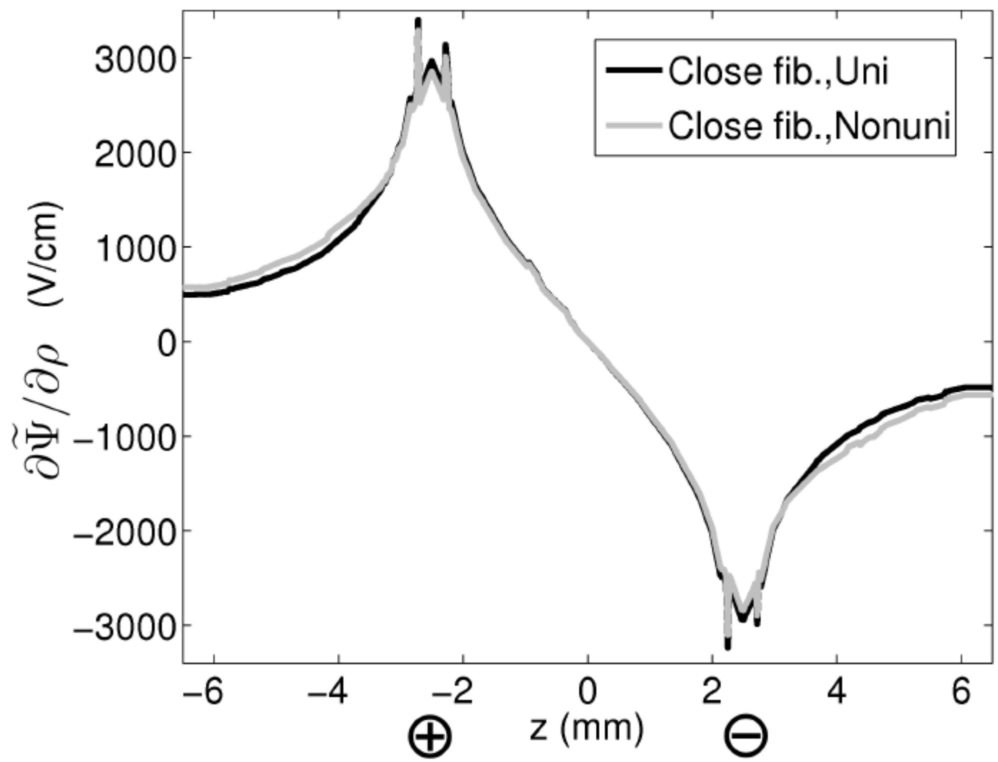
Total uptake: nonuniform version predicts 8.3% more uptake than uniform version

Mechanism

- Both AFs larger in magnitude near ends of fiber at $z = \pm 6$ mm
- Bot AFs smaller in magnitude near electrodes

→ "Smears out" AFs

Transverse AF



# Model Variations

## Effect of electroporation on tissue conductivities

Total uptake: nonuniform version predicts 8.3% more uptake than uniform version

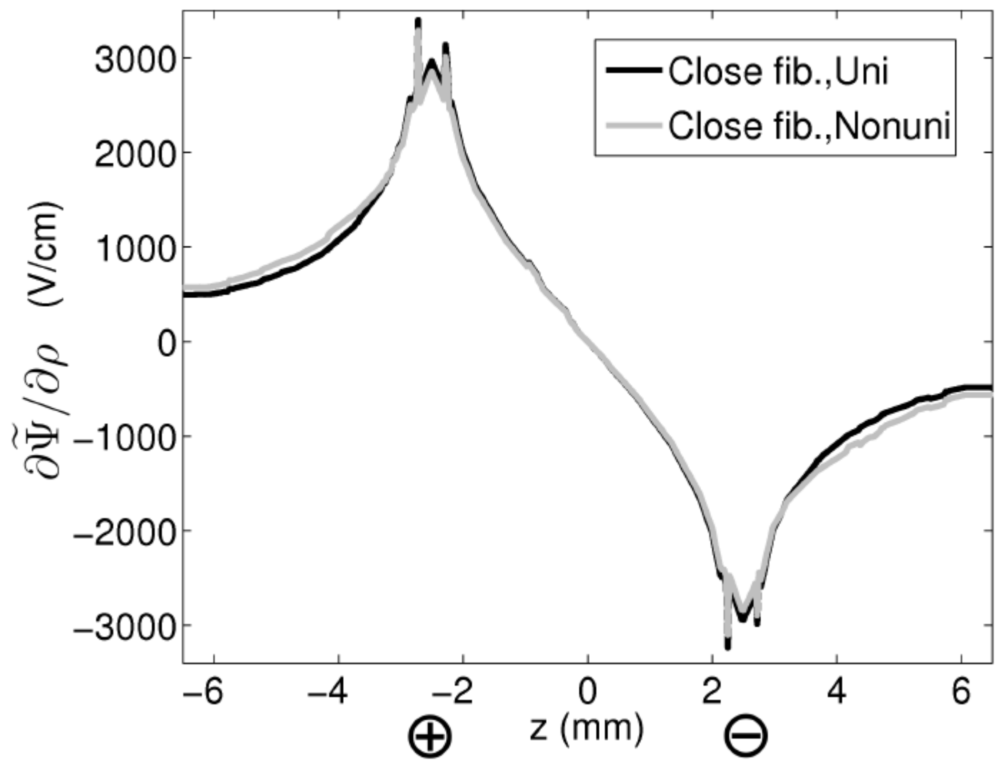
Mechanism

- Both AFs larger in magnitude near ends of fiber at $z = \pm 6$ mm
- Bot AFs smaller in magnitude near electrodes

→ "Smears out" AFs

30% difference in total tissue uptake using numerical AFs versus analytical AFs

Transverse AF



# Conclusions

Increase uptake when magnitude of AFs more evenly distributed over tissue

Reproduce tissue uptake experiments qualitatively and quantitatively

Importance of longitudinal AF not sensitive to changes in macroscopic tissue conductivities

Computationally efficient tool for 3D simulation

- New transverse AF
- Transverse and longitudinal electrical properties nerve stimulation

# Limitations and Future Directions

Electroporation parameters and dynamics

- Artificial lipid bilayer parameters underestimates EP
- Growing pores

Periodicity of fibers

- Fiber bending
- Between tendons
- Site of electrodes

# Limitations and Future Directions

Changes in macroscopic tissue conductivities due to EP

- Numerically solve additional 3D elliptic equation every time step
- Numerical error in AFs

Growing pores and DNA

- Large pores occur on “edges” of electroporated region
- Greater effect from longitudinal AF
  - Mechanism seen near ends of fiber
  - DNA can only pass through large pores

# Einstein Relation

$$D = \frac{uRT}{|z_m| F} = \frac{u(N_A k_B)T}{|z_m|(eN_A)} = \frac{u k_B T}{|z_m| e}$$

Electrical mobility is velocity/field, here for intra. space: $u = \boldsymbol{v_i} / E_i$

$$D = \frac{\boldsymbol{v_i} k_B T}{E_i z_m e}$$

(don't need $|z_m|$ anymore b/c define it so always positive as v/E)

$$\boldsymbol{v_i} = \frac{D z_m e}{k_B T} E_i = -\frac{D z_m e}{k_B T} \nabla V_i$$

(velocity due to electric drift)

# Stokes-Einstein Equation Diffusion Coefficient

$D = k_B T / 6\pi\mu a$ , $\mu$ is viscosity, and a is radius of molecule approx. as spherical

$a = (3\, MW / 4\pi\rho N_A)^{1/3}$ , where MW is molecular weight, p is density, N_A is Avogadro's #

$a = (3 * 400\ g/mol / 4\pi 9.932x10^{5}\ g/m\char`^3 * 6.022x10^{23}\ /mol)^{1/3} = 5.42x10^{-10}\ m$

$D = 1.38x10^{-23}\ J/K * 310\ K / 6\pi * 0.6965x10^{-3}\ N\,s/m^{2}\ \ 5.42597x10^{-10}\ m$ =6.0054x10^-10

D/3 b/c molecule not in water, it is in intra/extra space, shown to be 1/3 diffusion as in water: 2.01x10$^{-10}$ m^2/s

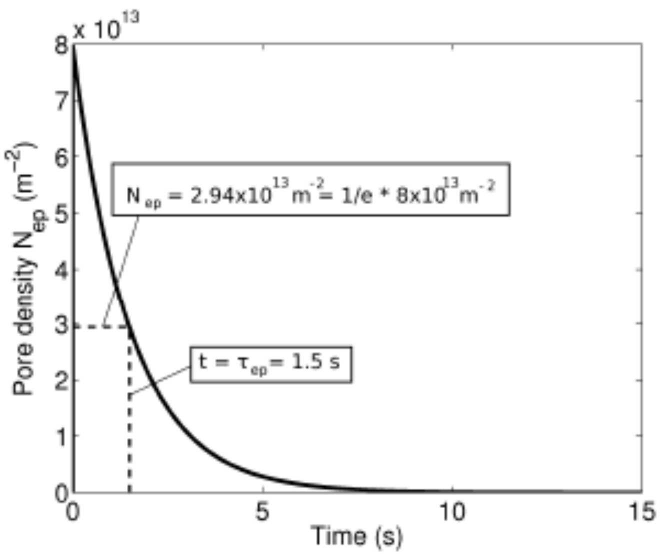
# Time Scale of Diffusion

$$\frac{dN_{ep}}{dt} = \alpha e^{(\Phi_m/V_{ep})^2}\left(1 - \frac{N}{N_0 e^{q(\Phi_m/V_{ep})^2}}\right)$$

$$\tau_{ep} = \frac{N_0}{\alpha} e^{(q-1)(\Phi_m/V_{ep})^2}\Big|_{\Phi_m=0} = 1.5 \text{ s}$$

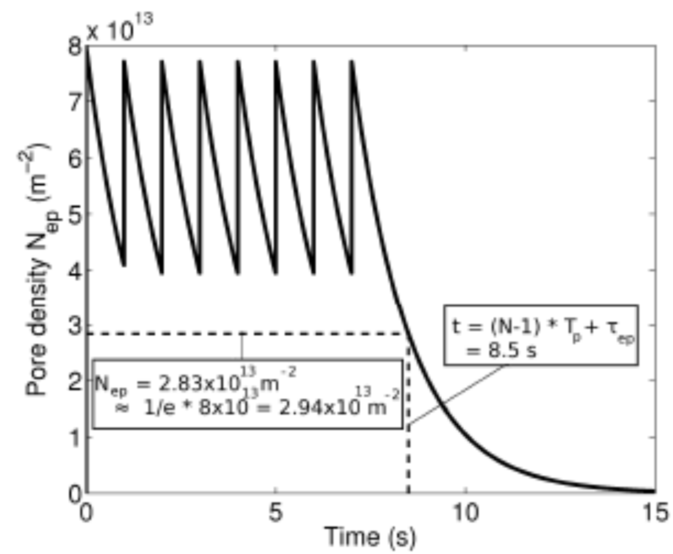
Pore Density
Single Pulse

(A)




Pore Density
Multiple Pulses

(B)



# Radioactivity

$$A_{Bq} = \frac{m}{m_a} N_A \frac{\ln(2)}{t_{1/2}}$$

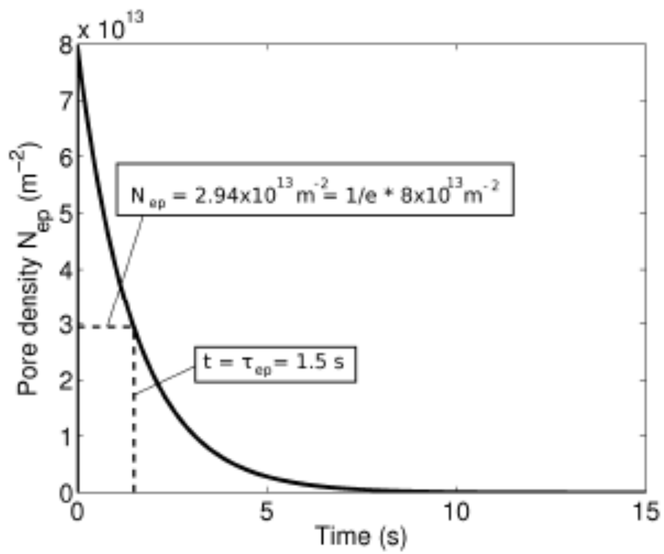
# Time Scale of Diffusion

$$\frac{dN_{ep}}{dt} = \alpha e^{(\Phi_m/V_{ep})^2} \left(1 - \frac{N}{N_0 e^{q(\Phi_m/V_{ep})^2}}\right)$$

$$\tau_{ep} = \left.\frac{N_0}{\alpha} e^{(q-1)(\Phi_m/V_{ep})^2}\right|_{\Phi_m=0} = 1.5 \text{ s}$$

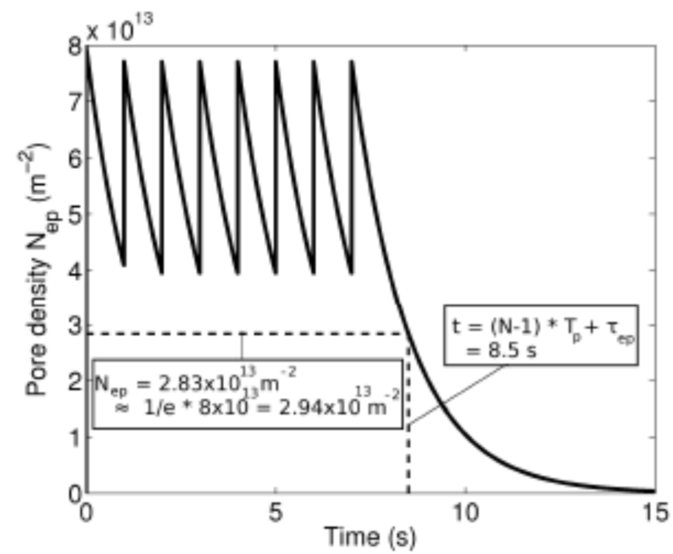
Pore Density
Single Pulse

(A)




Pore Density
Multiple Pulses

(B)



# Time and Space Scales in Longitudinal Equation

## 1D longitudinal Problem for mean potential: Passive membrane

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t}=\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}+\left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\langle\Psi\rangle}{\partial z^2}-\left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

Substitute: $$\overline{I_m}=\left(\frac{1}{R_m}\right)f_m^0-\frac{V_{rest}}{R_m}$$

$$\underbrace{\{R_mC_m\}}_{Timescale}\frac{\partial f_m^0}{\partial t}=\underbrace{\left\{\left(\frac{1}{1+\frac{1}{\gamma\mu}}\right)\frac{R_m\sigma_i a}{2}\right\}}_{\lambda^2}\frac{\partial^2 f_m^0}{\partial z^2}+\left\{\left(\frac{\mu(\gamma+1)}{\gamma\mu+1}\right)\frac{R_m\sigma_i a}{2}\right\}\frac{\partial^2\langle\Psi\rangle}{\partial z^2}-f_m^0-V_{rest}$$

$$Timescale=10\text{ ms}$$

$$\lambda=1\text{ mm}$$

Lesson: term in parentheses reduces length constant for tightly packed fibers, so that it is reasonable to place no-flux BCs at ends of fiber, which are 4 mm away from respective electrodes for 13 mm fiber and electrodes at +/- 2.5 mm. But for fiber in infinite medium, need longer fiber (24 mm) b/c length constant is $\sqrt{R_m\sigma_i a/2}=2.6\text{ mm}$

# Time and Space Scales in Longitudinal Equation

1D longitudinal Problem for mean potential: EPing membrane

$$\left(1+\frac{1}{\gamma\mu}\right)C_m\frac{\partial f_m^0}{\partial t}=\frac{\sigma_i a}{2}\frac{\partial^2 f_m^0}{\partial z^2}+\left(1+\frac{1}{\gamma}\right)\frac{\sigma_i a}{2}\frac{\partial^2\langle\Psi\rangle}{\partial z^2}-\left(1+\frac{1}{\gamma\mu}\right)\overline{I_m}$$

Substitute: $$\overline{I_m}=\left(\frac{R_m+R_p}{R_m R_p}\right)f_m^0-\frac{V_{rest}}{R_m}$$

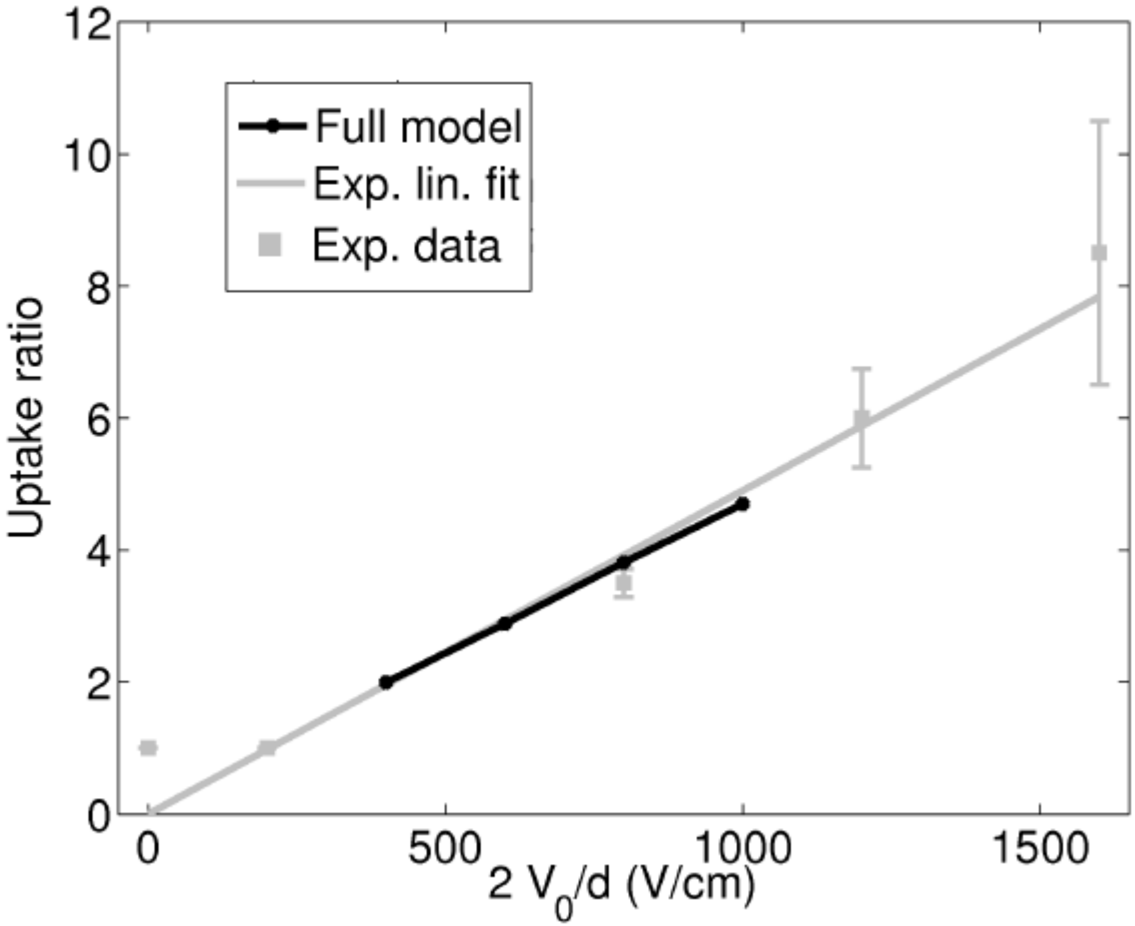
$$\underbrace{\left\{\frac{R_p R_m C_m}{R_m+R_p}\right\}}_{Timescale}\frac{\partial f_m^0}{\partial t}=\underbrace{\left\{\left(\frac{1}{1+\frac{1}{\gamma\mu}}\right)\frac{R_m R_p}{R_m+R_p}\frac{\sigma_i a}{2}\right\}}_{\lambda^2}\frac{\partial^2 f_m^0}{\partial z^2}+\left\{\left(\frac{\mu(\gamma+1)}{\gamma\mu+1}\right)\frac{R_m R_p}{R_m+R_p}\frac{\sigma_i a}{2}\right\}\frac{\partial^2\langle\Psi\rangle}{\partial z^2}-f_m^0-\frac{R_m R_p}{R_m(R_m+R_p)}V_{rest}$$

$$Timescale = 1\ \mu\text{s}$$

$$\lambda = 1\text{x}10^{\text{-}11}\ \text{m}$$

# Model Variations

<u>Pulse strength: comparison to experiments</u>




Linear correlation coefficient

- Model: 0.99996
- Experiments: 0.98

Relative difference in uptake never exceeds 5%

Experimental variability: 6%

Maximum contribution from longitudinal AF: 10%

# Model Variations

## Effect of electroporation on tissue conductivities

Simulate two versions solving $\Psi$ numerically

1. Uniform tissue conductivities
2. Nonuniform tissue conductivities – not change sigmaz, sigmay 2.5x larger still

Find numerical transverse AF $\partial\tilde{\Psi}/\partial\rho$ and longitudinal AF $\partial^2 \langle \Psi \rangle / \partial z^2$

Nonuniform version

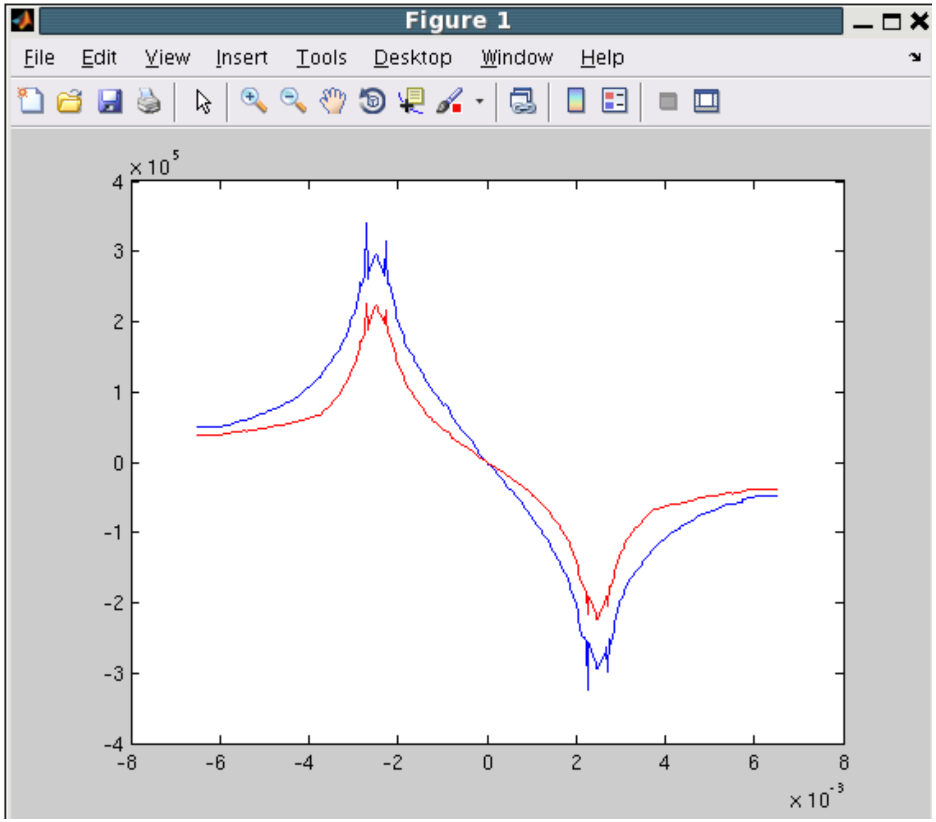
6.7% increase in uptake from longitudinal AF

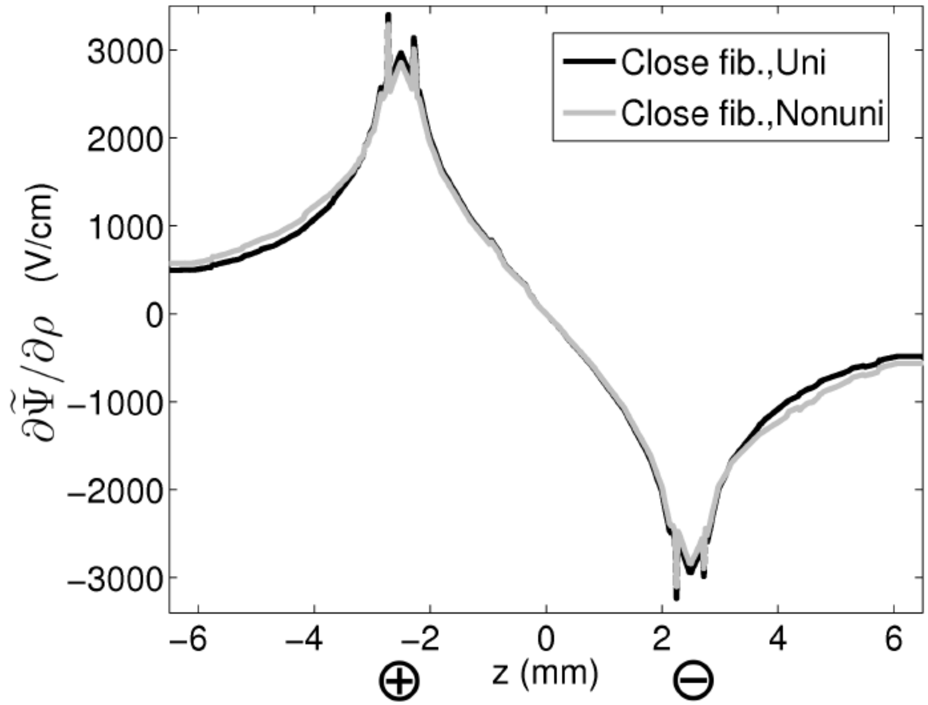
Uniform version

3.77% increase in uptake from longitudinal AF

Neglecting effect of EP on tissue conductivities does not change qualitative role of longitudinal AF much

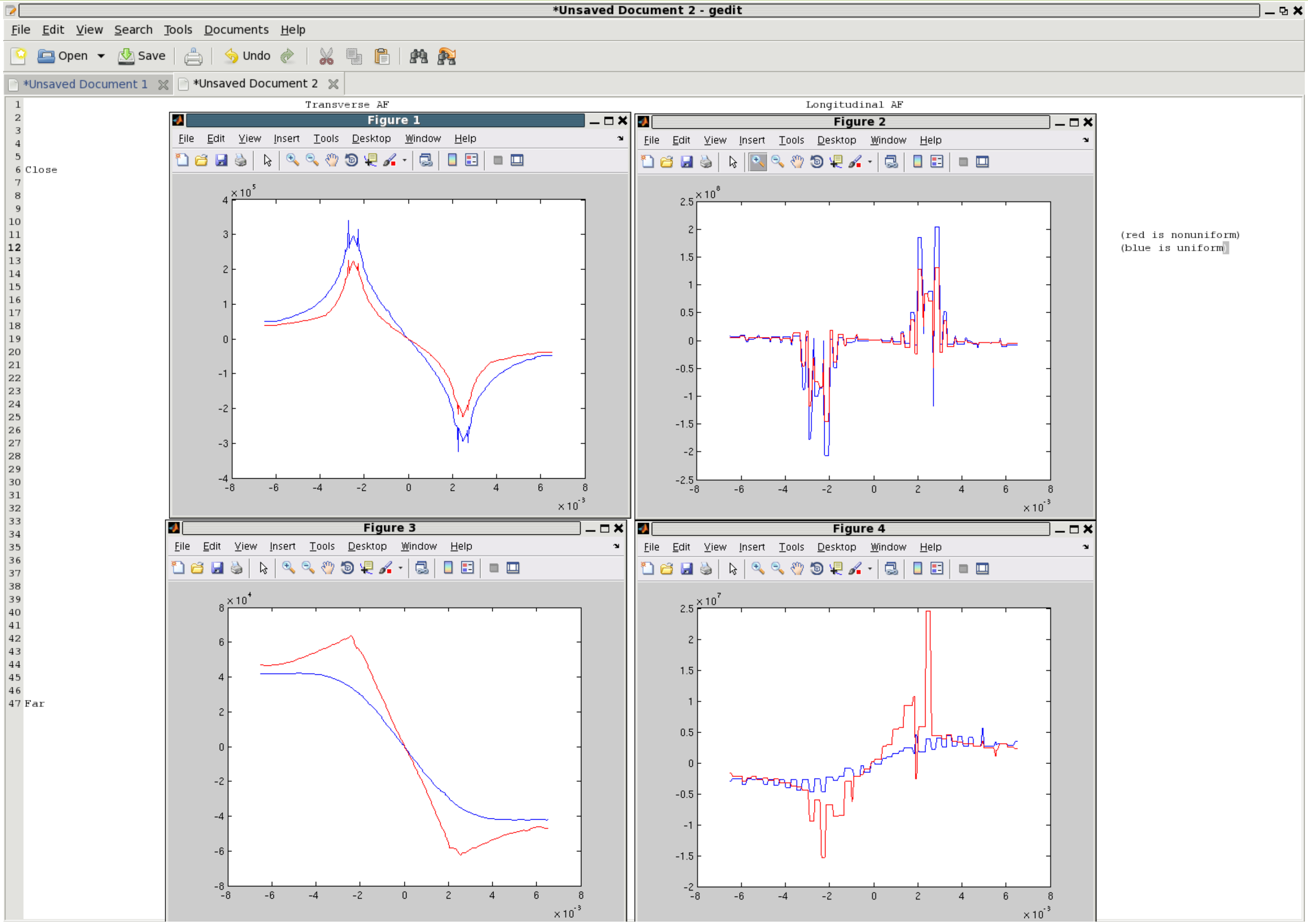
# Model Variations



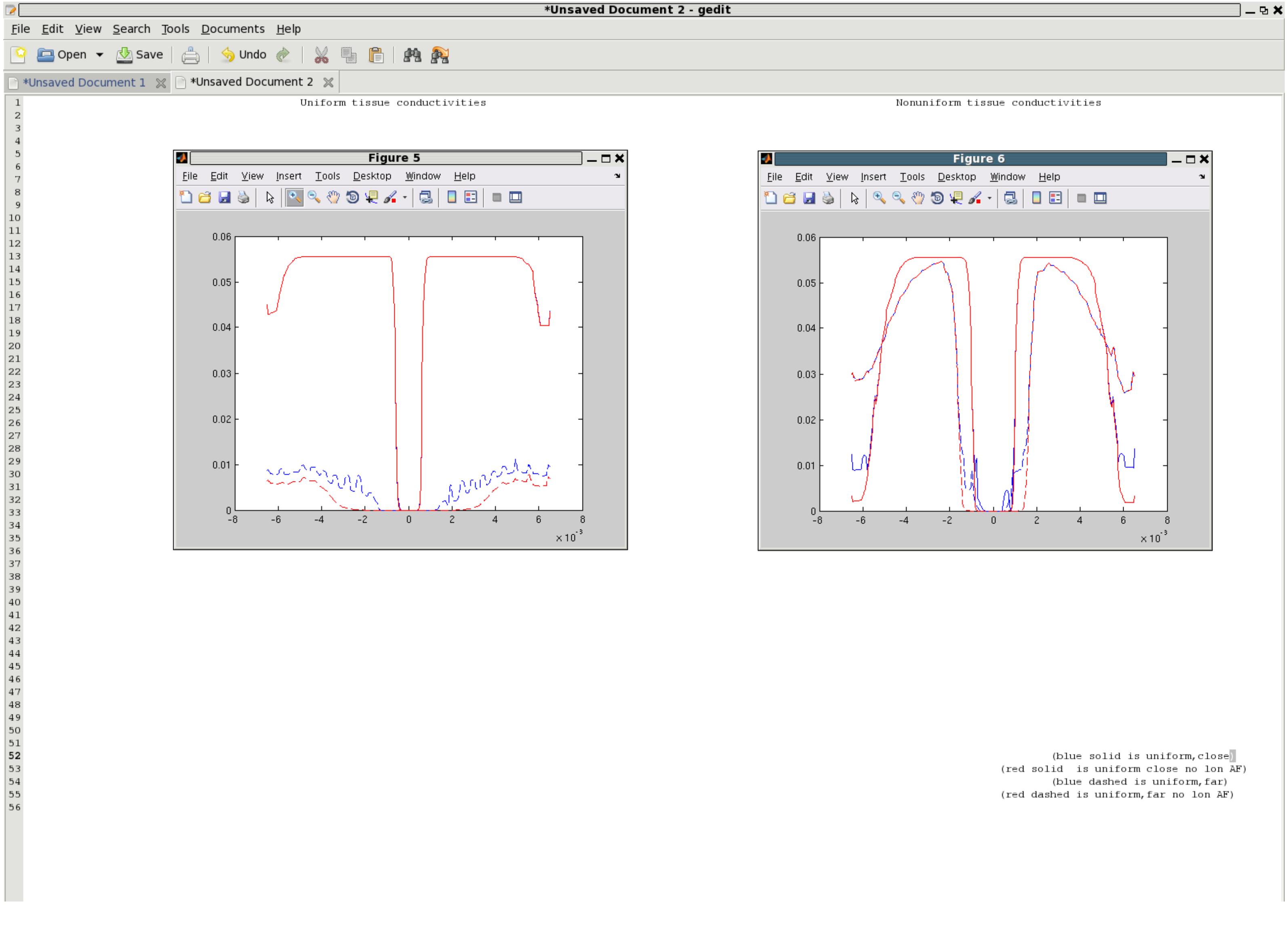




Reason is, for fiber close to electrodes, transverse AF is smaller near ends of fiber, which allows lon. AF to charge and EP membrane more. Whereas when both conductivities increase the trans. AF is larger near ends, which prevents charging via lon. AF

# Model Variations



# Model Variations



# Broader Significance

Current models of neurons

1. Solely longitudinal charging of membrane
   - Not as accurate for close fibers
2. Longitudinal charging, with constant term for transverse charging
   - Not as accurate for close fibers
3. Solve 3D BVP for longitudinal and transverse charging
   - Computationally expensive

Advantages of asymptotic fiber model

- Less expensive
- Accounts for **dynamic** effect of combined transverse and longitudinal charging

Acknowledgements

## Thank you

Dr. Wanda K. Neu

Dr. Roger C. Barr
Dr. Thomas J. McIntosh
Dr. Thomas P. Witelski
Dr. Fan Yuan

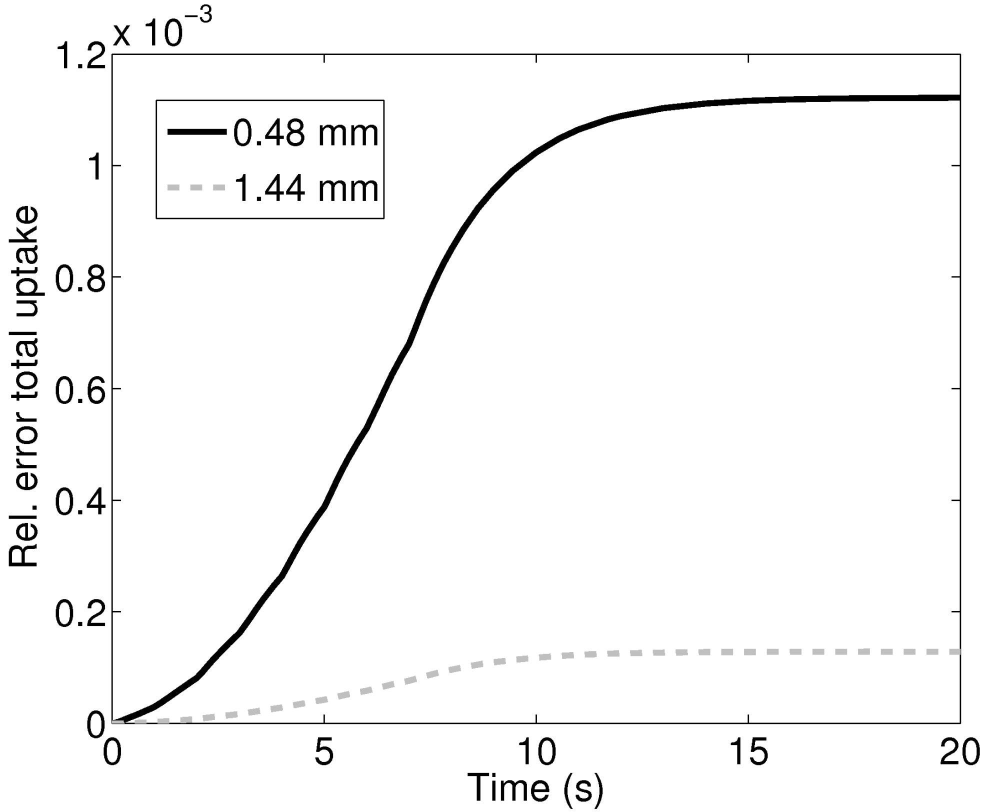
### Other colleagues and mentors

Dr. Margot Bowen
Dr. James Esterline
Dr. Mazella B. Fuller
Dr. Ann Pitruzzello
Caroline Ring
Dr. John Wambaugh

# Description of Model Components

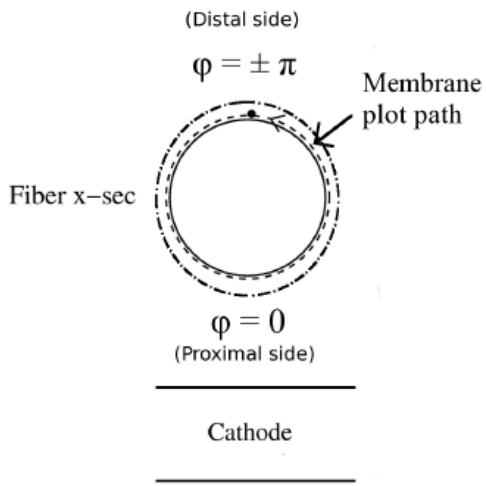
## 4. Mass transport

- Justification for using series of longitudinally-independent problems



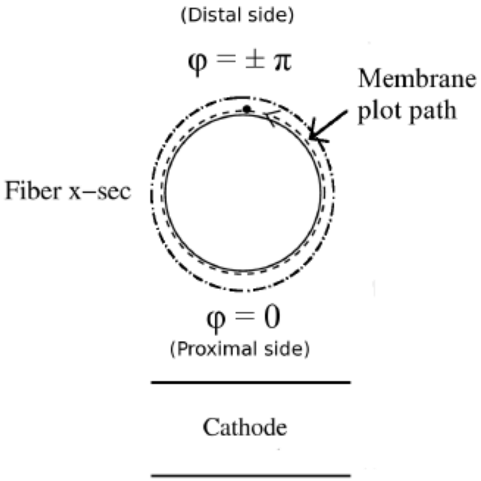
# Passive Versus Electroporating

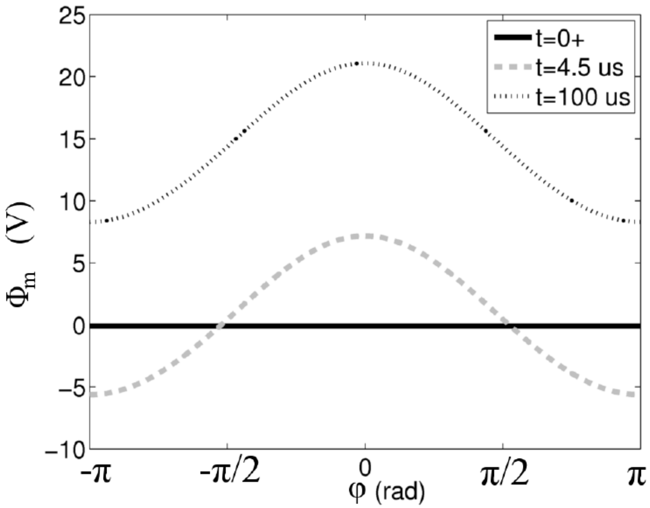
## Circumferential Transmembrane Potential



# Passive Versus Electroporating

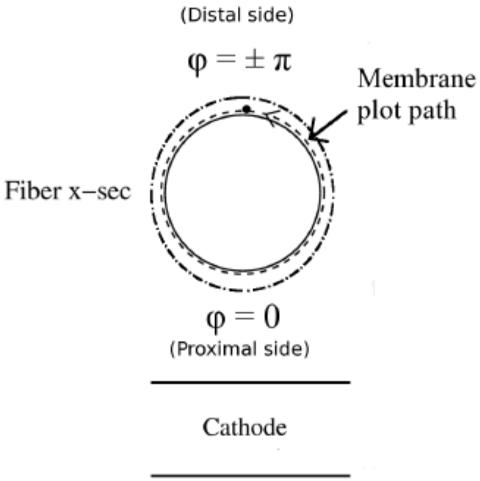
## <u>Circumferential Transmembrane Potential</u>

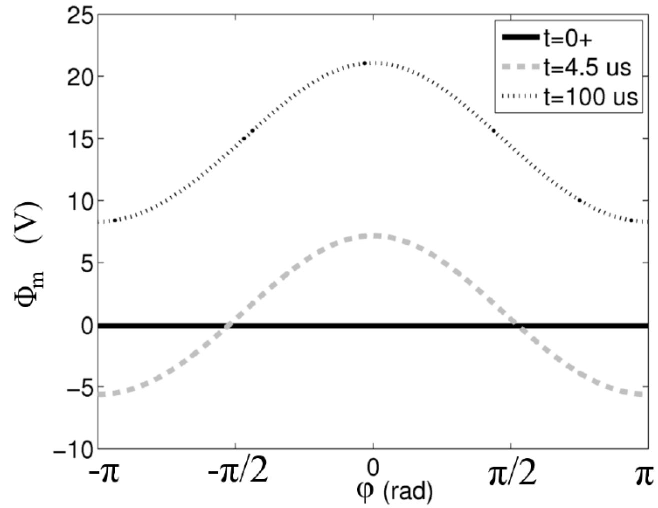


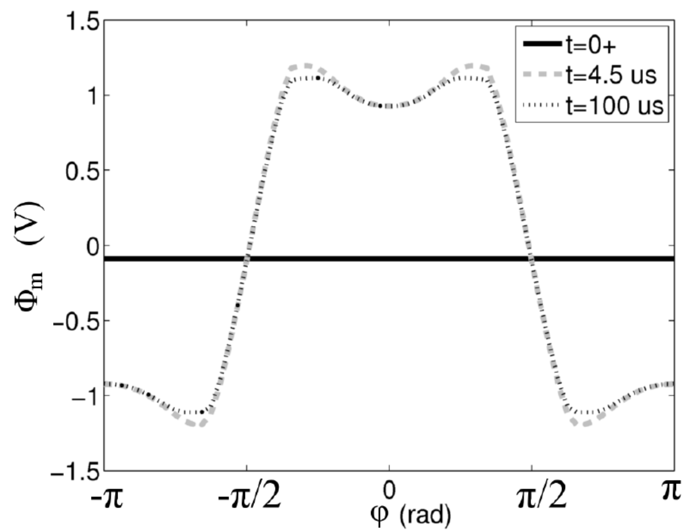



# Passive Versus Electroporating

## Circumferential Transmembrane Potential

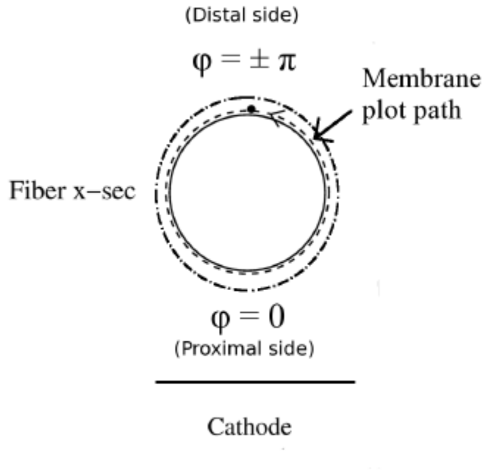


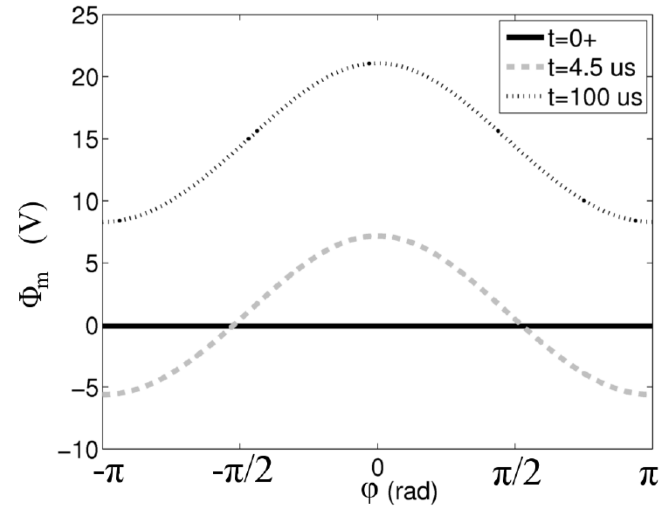



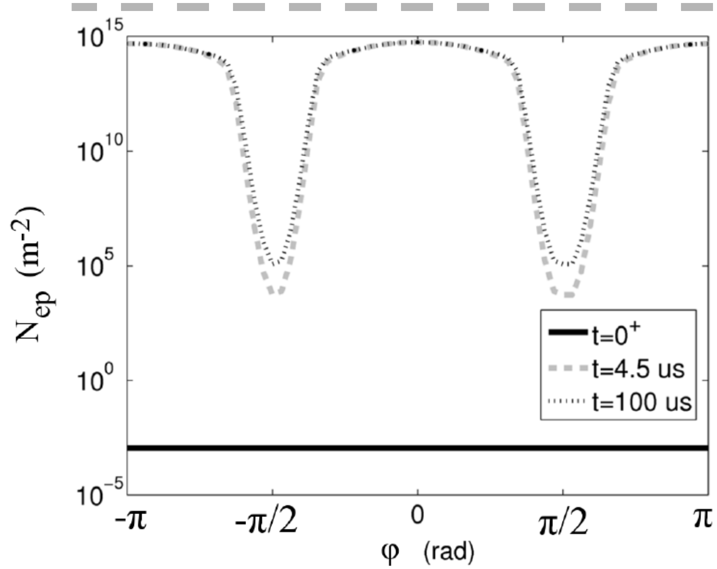



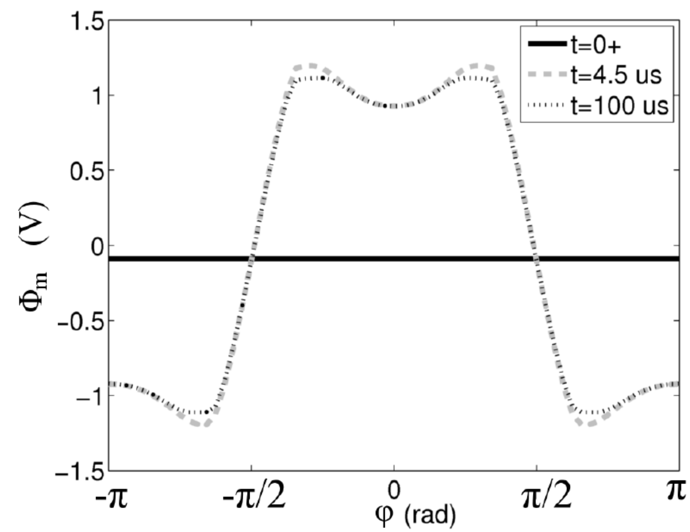
# Passive Versus Electroporating

## <u>Circumferential Transmembrane Potential</u>



# Broader Significance

Difficulties in modeling DNA uptake

- DNA may have two order magnitude small diffusion constant, so may not be able to assume well-mixed in transverse direction
- DNA may form complexes with the membrane before crossing membrane
- May involve endocytosis

# Broader Significance

Extension to DNA uptake

- pDNA shown to move micrometers during pulses, pore density varies longitudinally on order of millimeters
- Series of longitudinally independent problems may still hold validity
- $N_{ep}$ along length of fibers determines uptake across membrane
- $N_{ep}$ found from asymptotic fiber model, independent of uptake,
- Uptake across membrane may be similar, but just scaled by slower diffusion coefficient $D_0$, retaining same dependency on longitudinal charging

$$\partial c_i / \partial t \propto D(c_e - c_i) N_{ep}$$

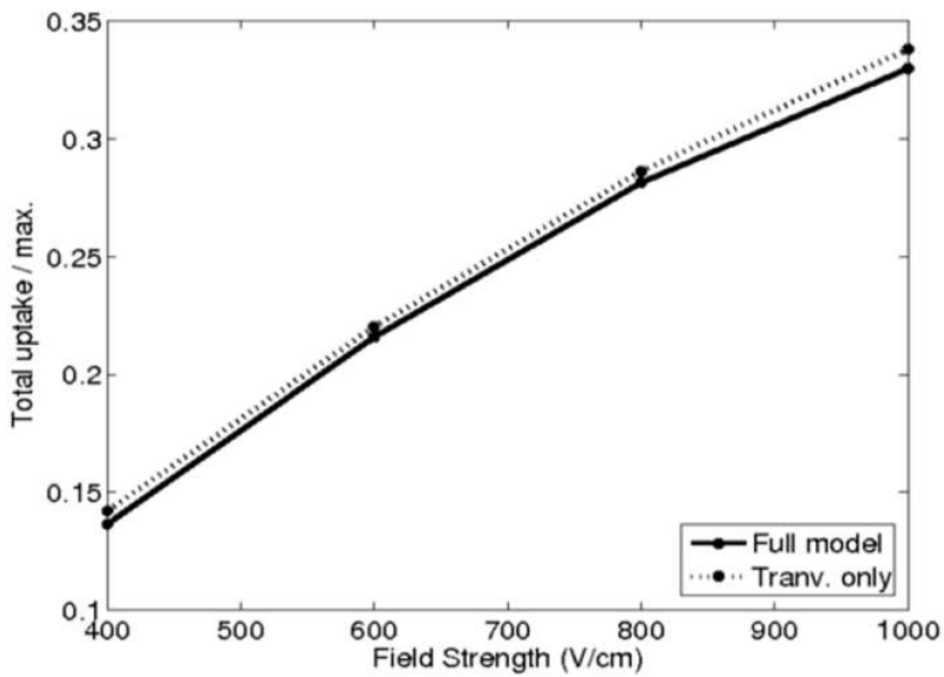
$$\partial c_e / \partial t \propto D(c_i - c_e) N_{ep}$$

# Proposed Additions

I. Error from neglecting longitudinal AF in tissue-wide uptake as vary
   1) Pulse strength
   2) Pulse duration

      Interesting – no further membrane charging for fibers close to electrodes

   3) Electrode orientation (longitudinal versus transverse)



# Proposed Additions

II. Compare tissue-wide uptake results in I. to results from experiments and other models of electroporation-mediated molecular uptake